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CONTENTS.

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ON THE DEVELOPMENT OF THE LYMPHATICS IN THE STOMACH OF THE EMBRYO PIG.

By James R. Cash,

From the Anatomical Laboratory, Johns Hopkins University.

With three plates and three text-figures.

ON THE DEVELOPMENT OF THE LYMPHATICS IN THE STOMACH OF THE EMBRYO PIG.

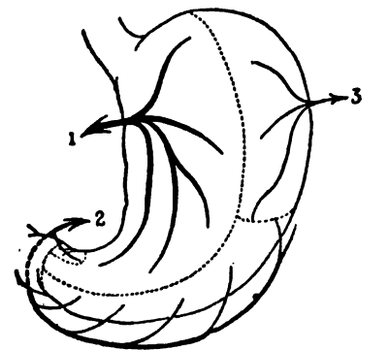
The work of Dr. Mall (1896) on the anatomy of the lymphatics of the wall of the stomach has established the plexiform arrangement of these vessels and their relation to the other structures of this organ. By dissecting the stomachs of dogs in which the lymphatics had been injected, and by reconstructions of such dissections, the entire organ was shown to be supplied with four definite, homogeneous plexuses. The most delicate of these, and the one most intimately related to the lining of the stomach, is the mucosal plexus. It lies in the mucosa at the base of the gastric glands just external to the muscularis mucosæ and receives branches which lie between the glands and extend out as far as the gastric pits. Owing to the delicacy of these vessels and the fact that valves prevent their injection from the main plexus of the submucosa, any attempt at complete injection is attended with much difficulty. As a matter of fact, the mucosal vessels can be injected in the adult only by direct puncture, which means that the needle must actually pass through the muscularis mucosæ and enter the narrow zone between it and the base of the gland; it can be readily seen that such an exact placing of the needle occurs only by chance. However, the best specimens obtainable indicate clearly that the lymphatics between the glands communicate directly with this delicate, homogeneous plexus lying at the base of the glands of the mucosa.

Proceeding through the wall of the stomach toward its serous surface, the first plexus of the submucosa is encountered. This consists of a single layer of lymphatics, just outside the muscularis mucosæ, and forms the connecting link between the mucosal plexus, just described, and the main plexus of the submucosa, the principal plexus of the wall of the stomach. This main plexus is a dense mass of vessels which practically fills the submucosa and is composed of several layers of large, tortuous, thickly arranged lymphatics. Springing from it are numerous slender vessels which traverse the muscular coats of the stomach, forming a coarse plexus between them, and are gathered together on the surface of the stomach into the subserous plexus, a dense, single layer of lymphatics situated just beneath the peritoneal covering of the organ.

The work of Dr. Mall extended that of Sappey, Teichmann, and Loven. It may be seen, therefore, that the anatomy of the lymphatic vessels in the wall of the adult stomach is well known. The pathways of lymphatic drainage from this organ, however, are not so clearly understood. All investigations along this line have consisted in partial injections of the adult stomach. In the present experiments the direction of flow of the injection mass from different points was noted and from the information so gained the direction of normal lymphatic drainage, *in vivo*, is inferred.

The most extensive work of this kind has been done by Cuneo. By injecting colored substances directly into the main plexus of the submucosa, and watching its

flow over the wall of the stomach and its appearance in regional lymphatic glands, he has obtained a very good general idea of this question. As a result, he felt justified in dividing the stomach into three main lymphatic zones (Poirier and Cuneo, 1902). These arbitrary zones are shown in text-figure 1. The arrows show the direction of the flow of lymph from the different parts. Zones 1 and 2 drain the glands of the lesser curvature, while the flow from zone 3 goes to the hilum of the spleen and then on to the pre-aortic glands. It is to be noted that no drainage is indicated by way of the duodenum. Cuneo states that most of the lymph flows directly toward the lesser curvature and passes through glands situated in this region, whose efferent ducts drain into the pre-aortic lymph-nodes just anterior to the cysterna chyli. Zone 1 represents this area. In the area designated as zone 2 the flow of lymph is toward the pylorus, as indicated, but passes posterior to it to the glands of the lesser curvature. From zone 3 lymph passes through vessels in related folds of peritoneum, to the hilum of the spleen and from there to the pre-aortic lymph-nodes.



TEXT-FIG. 1.—Lymphatic territories of the stomach. After Cuneo, 1902.
1. The coronary or principal current.
2. Right gastro-epiploic current.
3. Splenic current.

As one looks at a cross-section of a stomach wall in which the lymphatics have been injected, the extreme vascularity of the organ becomes immediately apparent. The plexuses are so dense, permeate the entire organ so thoroughly, and are so rich in their anastomoses that definite lymphatic zones and fixed points of drainage for the different regions are anatomically difficult to establish. The more conservative and apparently more accurate view is that the wall of the stomach contains four complete lymphatic plexuses, in open communication with each other and through which lymph may flow, peripherally, in any direction. It is quite difficult to conceive of such dense, homogeneous plexuses being divided into any zone-like arrangement. Certainly, the lymph will leave the stomach by way of the nearest and most accessible avenue of exit, and injections made around the regions toward which lymphatic flow occurs will apparently find zones in which most of the flow takes place in a given direction; but in view of the complex nature of the vessels within the wall of the stomach the accuracy of areas found in this manner becomes very doubtful. Thus it is evident that our knowledge of the gastric lymphatics is not yet complete. Such methods as those mentioned are sufficient only for the formation of general ideas and beyond this point any statement is speculative. As yet, no work has been done on the embryology of these vessels. To resolve such dense lymphatic plexuses into their component parts by injection after they are fully developed is obviously impractical. By a study of their origin and development it was hoped that more accurate views might be adopted for the lymphatic drainage of this organ, and it was with this idea that the present investigations in embryo pigs were undertaken.

By the method of injection, embryos can be studied in various stages before the lymphatic development is complete. In this way the origin of the vessels, the

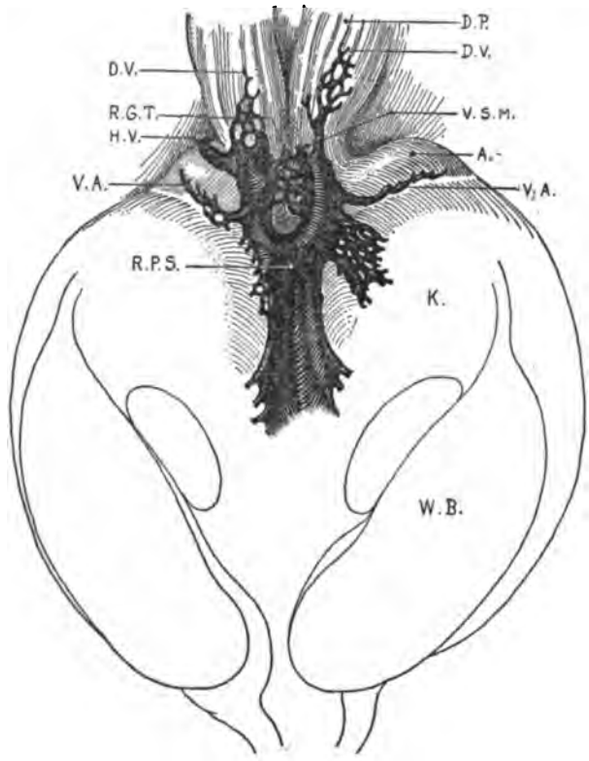
points at which they reach the stomach, their manner of growth, their anastomoses, and finally the relation of the gastric lymphatics to the lymphatics of other organs can be observed. Embryos 40, 60, 80, and 120 mm. in length were used. Those up to 90 mm. were injected through the retroperitoneal sac, or its remains, the large lymphatic plexus of vessels between the Wolffian bodies. In the embryos measuring over 90 mm. the injections were made directly into the submucosal plexus at the lesser curvature, since valves and nodes, forming at this age, prevent retrograde injection of the organ from the retroperitoneal sac. In such a study it would be very helpful to have complete injections of the lymphatics of the adult stomach and its surrounding organs, but the valves and nodes along the course of the vessels act as effective barriers to extensive injections. For a successful injection the embryo must be fresh, preferably with the heart still beating. Hypodermic syringes of 1 c. c. capacity with fine needles (No 28), were used for injecting. The best specimens were obtained with a saturated aqueous solution of Berlin blue as the injection mass. Most of the material was cleared in oil of wintergreen by the Spalteholz method. Thick microscopic sections were cut to show the progressive development of the plexuses within the stomach wall and the course of the vessels which traverse the ligaments of the spleen. The actual technique of injecting the retroperitoneal sac has been described by Baetjer (1908) in his work on the morphology of that structure and need not be repeated here.

In their development the lymphatics of the stomach pass to it by way of its related folds of peritoneum. Therefore, a clear idea of the relation of these folds to the stomach, as well as to the points of origin of the lymphatic trunks which invade it, is imperative for an understanding of the development of these vessels. As the relations of this part of the peritoneum in the pig are slightly different from those in the human body, a brief description of them is necessary. The dorsal mesogastrium which later forms the great omentum, contains the spleen between its layers. The spleen lies ventralward, close along the greater curvature of the stomach, and has two ligaments. The first of these is the gastro-splenic, that part of the omentum connecting the spleen with the greater curvature of the stomach. The second is the splenic ligament, that part of the omentum which forms the posterior wall of the omental bursa and from the hilum of the spleen is continuous with the peritoneum covering the Wolffian body, the general mesentery of the intestine and the mesentery of the duodenum. The transverse colon, with its mesentery, lies free from the omentum. The duodenum has a broad, fan-shaped mesentery which continues cephalad to that part of the omentum forming the posterior wall of the omental bursa and caudad to the common mesentery of the small intestine. The ventral mesentery remains as the gastro-hepatic ligament, connecting in the usual manner the lesser curvature of the stomach with the hilum of the liver.

The retroperitoneal sac, discovered by F. T. Lewis, has been well described by Sabin, Baetjer, Heuer, and other workers in this laboratory. It is a triangular structure which lies at the base of the mesentery at the level of the cœliac axis. There are two small indentations along its sides, made by the adrenal bodies, which thus divide the sac into two lobes. From the posterior lobe, which is the larger,

arise the lymphatics of the intestine and of the abdominal structures, while from the anterior lobe spring vessels to the stomach, spleen, duodenum, diaphragm, and lungs (text-figure 2). It is this anterior portion of the sac with which we are primarily concerned in this study. The entire sac is obliterated during development, being the primordium for the chain of lymph-glands which reach from the cœliac axis to the bifurcation of the aorta.

The gastric vessels arise by two main trunks from the anterior lobe of the retroperitoneal sac. The right trunk is much larger than the left and passes behind the stomach to the lesser curvature, where it invades the stomach at three different



TEXT-FIG. 2.—Diagram of the retroperitoneal sac showing the relation of the gastric trunks as they leave its anterior end. The early lymphatics extending to the pillars of the diaphragm are also illustrated.

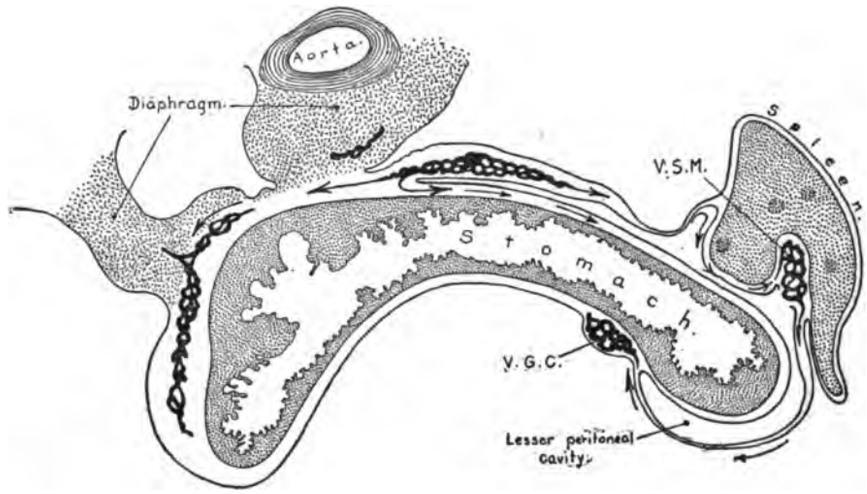
A., Adrenal; *D. P.*, pillar of the diaphragm; *D. V.*, diaphragmatic vessels; *H. V.*, hepatic vessels; *K.*, kidney; *R. G. T.*, right gastric trunk; *R. P. S.*, retroperitoneal sac; *V. S. M.*, vessels of splenic mesentery; *W. B.*, Wolffian body.

points: (1) the opening of the esophagus, (2) the center of the lesser curvature, and (3) the pyloric extremity of the lesser curvature. These three sets of vessels to the stomach are well shown by a comparison of figures 6 and 7. It can be seen (fig. 6) that another vessel of considerable size is given off from the right trunk, or arises as a separate trunk from the anterior part of the retroperitoneal sac, and passes through the mesentery to the duodenum (A. Duo. V.). It then extends along the duodenum to the pylorus, where it anastomoses freely with the pyloric vessels, reaching the stomach from its posterior surface (fig. 4).

The left gastric trunk, or splenic trunk, as it may be called from its distribution, divides into two branches. One passes directly to the cardiac pouch and is not shown in the figures; the other passes through the splenic ligament to the hilum of the spleen, the interior of which it has never been seen to enter, and then

traverses the gastro-splenic ligament to the center of the greater curvature of the stomach where it ramifies to right and left (fig. 4). These lymphatics then anastomose, over both the anterior and the posterior walls, with those from the lesser curvature, where connections are formed with the lymphatics of the esophagus and duodenum (cf. figures 4 and 5, plate 1).

After this brief description of the general pathways along which lymphatic invasion of the stomach takes place, the characteristics of each invading set of vessels and their part in forming the general lymphatic plexuses remain to be considered.



TEXT-FIG. 3.—Pig embryo, 100 mm. long. Diagrammatic transverse section of the stomach and spleen with their peritoneal ligaments. The arrows which begin from the retroperitoneal sac show the directions in which lymphatic invasion of the stomach takes place. In this specimen the stomach is collapsed. The hilum of the spleen is marked by vessels of the splenic mesentery (V. S. M.); also the greater curvature of the stomach may be located both by its vessels (V. G. C.) cut in cross-section and by the attachment of the gastro-splenic ligament.

The stomach may be regarded as being invaded from the posterior side at the lesser curvature by the right trunk, and anteriorly along the greater curvature by the left trunk. The posterior invasion of the lesser curvature takes place along the gastro-hepatic ligament (the primitive ventral mesentery), while the anterior invasion at the greater curvature is by way of the splenic and gastro-splenic ligaments (the primitive dorsal mesentery of the stomach) and the mesentery of the duodenum (text-figure 3). This arrangement offers a striking contrast to the manner in which the lymphatics reach the intestine. Here, straight trunks grow out from the retroperitoneal sac through the mesentery and, on reaching the wall of the intestine, divide into right and left branches to surround the gut. In the case of the stomach the right and left branches leave the retroperitoneal sac directly and pass to the stomach by way of two separate folds of related peritoneum.

The earliest stage injected was an embryo 28 mm. in length (fig. 7). The vessels arising from the retroperitoneal sac can be seen just reaching the stomach. This is a very early stage and the injected vessels are those arising from the right gastric trunk of the retroperitoneal sac. In this specimen the spleen and liver have been removed. It has never been possible to inject the splenic vessels (left gastric trunk) at this stage, but the mode of entrance of the right gastric trunk is well illustrated. Figure 7 shows the primitive mass of lymphatics which later becomes grouped into the right gastric trunk and distributed to the stomach in three parts:

at the esophageal opening, at the center of the lesser curvature, and at the pylorus. It is from this mass of lymphatics that most of the gastric vessels develop. It will be noted in the figure that the lymphatics to the esophageal opening already suggest the ring which is later to become so characteristic. The central mass to the lesser curvature can be seen emerging from behind the hepatic vessels which are cut off in the drawing. These vessels to the lesser curvature are shown spreading out over the anterior surface of the stomach; they also extend over the corresponding area on the posterior wall. Two or three small vessels (P. V.) pass forward to the pylorus and it is important to note that they always reach this structure at its posterior wall. It is these vessels which will later anastomose with both the ascending duodenal vessels (A. Duo. V.) and the vessels of the greater curvature (V. G. C.), as shown in figure 4.

Growth is very rapid and in slightly older specimens (35 to 45 mm.), in which the splenic vessels have also appeared, the rapid invasion from the lesser cuvature is apparent. The vessels reaching the esophagus have by this time formed a complete subserous, periesophageal ring. This ring continues to develop and in older specimens forms a dense, circular plexus around the esophagus, from which branches pass in all directions to ramify over the stomach wall (fig. 8). The annular structure of this plexus is not only manifest as a subserous set of vessels, but is maintained in the depths of the stomach wall in this region as development proceeds.

The vessels which reach the stomach at the center of the lesser curvature are by far the most numerous of any of the groups. As they traverse the gastro-hepatic ligament they are in direct communication with the lymphatics of the liver, which pass through this structure and also take origin from the retroperitoneal sac (fig. 2). On reaching the stomach they branch over both the anterior and posterior walls and form numerous anastomoses with the vessels from the esophageal ring, as well as with the lymphatics reaching the lesser curvature at the pylorus. These pyloric vessels form the smallest group of lymphatics invading the stomach. They spring from the right trunk just lateral to the large group at the center of the lesser curvature and pass through the gastro-hepatic ligament to the posterior surface of the pylorus, over which they anastomose freely with the set of vessels reaching the pylorus by way of the mesentery of the duodenum.

This latter set of lymphatics (fig. 4), to which we may refer as the ascending duodenal vessels, forms a trunk of considerable size. They can be injected only in the younger embryos, due to the fact that one or more lymph glands are later formed in the mesentery of the duodenum at the pyloro-duodenal junction. Their rich anastomosis with the other gastric vessels presents a subject of unusual interest, inasmuch as it is not generally stated that lymphatic drainage of the stomach takes place partly by way of the mesentery of the duodenum. The magnitude of early vessels invading the stomach from this source and the extent of their anastomoses with the gastric vessels proper give proof of a well-defined pathway by which lymphatic drainage from the stomach may take place. Figures 4 and 6 show the extent of the anastomosis of these ascending duodenal vessels with the other vessels of the pylorus.

The origin of the ascending duodenal group is somewhat varied in the different specimens. In some, these vessels spring from the right gastric trunk as it leaves the retroperitoneal sac; in others, they arise from the sac itself just posterior to the right gastric trunk. In their course through the duodenal mesentery they present no unusual features, but the lymph-glands, later formed along their course at the pyloro-duodenal junction, offer a striking point of difference from the glands of the other vessels leaving the stomach. These glands prevent the injection of the duodenal lymphatics from points on the stomach and this fact most likely accounts for the failure to recognize such a connection between gastric and duodenal lymphatics by methods such as have been hitherto employed to demonstrate them.

The left gastric trunk, arising from the retroperitoneal sac, immediately divides into two branches. The lesser branch passes by way of the extreme left portion of the omental bursa to the anterior surface of the stomach at the base of the cardiac pouch. The cardiac pouch is a transient, embryonic structure. In the pig, during embryonic life, there is a slight annular constriction of the stomach wall near the cardiac end, which gives to that portion of the stomach a pouch-like appearance (fig. 5). This structure becomes less evident as development proceeds and in very late stages is scarcely perceptible. The lesser branch of the left cardiac trunk is the principal source of invasion of the pouch and forms rich anastomoses with the vessels reaching the stomach at the center of the lesser curvature and esophageal opening. The greater branch of the left gastric trunk enters the omental bursa just medial to the lesser branch. It passes by way of the posterior wall of the omental bursa (which in this region constitutes the splenic ligament) to the hilum of the spleen; from this point these vessels pass by way of the gastro-splenic ligament to the center of the greater curvature of the stomach (figs. 4 and 5). The number of vessels comprising this set varies from two to four. It is always difficult to be certain of the exact number, as they are situated very close together in a densely entwined mass. They have never been observed to enter the spleen, and sections taken through the point where they pass the hilum of that organ have consistently failed to demonstrate a trace of injection mass within its interior. So it may be said that these vessels appear in no way related to the spleen itself but merely take advantage of its folds of peritoneum to make their way to the stomach. They reach that organ on the greater curvature, at a point about midway between the cardia and pylorus, and from this point their growth proceeds in opposite directions. Approximately half of them pass to the right, along the greater curvature, to meet the pyloric vessels from the duodenum which grow along this course; the other half pass along the greater curvature, to the left, where they meet anastomosing vessels from the cardia, esophageal ring, and lesser curvature (fig. 4). All along their course on the greater curvature these vessels give off branches at right angles to themselves, which extend over the anterior and posterior walls of the stomach to meet similar branches given off from the great mass of lymphatics of the lesser curvature.

Concomitant with the growth of the subserous trunks, growth of the other plexuses takes place. Sprouting branches from the early trunks of the subserosa

dip down into the stomach wall and, as the muscular layers are traversed, the plexus between the muscular layers is formed. This plexus extends throughout the stomach wall, but its vessels are of smaller caliber and its meshes much less dense than those of the other plexuses. It should be borne in mind, however, that vessels lying in dense, muscular tissue fill less readily with injection mass than those situated in the loose tissues of the submucosa and subserosa; therefore, the appearance of the muscular plexus may be less dense than it really is. This point considered, the small size of the vessels composing the plexus of the muscularis and their striking constancy of arrangement in all well injected specimens nevertheless are facts which tend to show that this plexus is a relatively scanty one.

On reaching the submucosa these vessels form the most extensive plexus of the stomach wall, the main plexus of the submucosa. Here lymphatic growth takes place very rapidly. At an early stage (20 to 40 mm.) an extremely dense plexus, several layers in thickness, composed of large, tortuous vessels, has been formed. In embryos 70 to 100 mm. in length the entire submucosa is found to be filled by this plexus. Its presence can be easily demonstrated throughout the entire stomach by injecting directly into the submucosa. As it nears the pylorus its vessels become smaller and many of them appear to end blindly in this region; however, a few of them have been seen to anastomose with those of the deep plexus of the duodenum. In view of the general tendency toward anastomosis exhibited by the lymphatics of contiguous tissue, which is especially marked in the lymphatics of the subserous plexuses of the stomach and duodenum, this scanty connection between the submucosal plexuses of these two organs can scarcely be considered as representing the true morphology of the lymphatics in this region. It is evident that the layers of the stomach wall at the pylorus are compressed by the tone of the pyloric musculature, as are also the lymphatic channels of the submucosa. Such a barrier proves quite effective in preventing complete injection of the vessels of this region. The character of the apparent endings of the vessels of the submucosa at the pylorus favors such a view. Instead of forming a complete plexus at this point, most of the vessels end blindly and at different points along the pylorus, thus giving the exact picture of an incomplete lymphatic injection elsewhere in the body. From the injections we are justified in saying only that the pathway between the submucous plexuses of the stomach and duodenum is not easily traversable during tonic contraction of the pylorus. A sufficient number of anastomoses between these two plexuses has been demonstrated to show that they are connected and, by analogy to the lymphatics of the rest of the gastro-intestinal tract, one would expect ready communication between them during relaxation of the pyloric sphincter.

The further growth of the lymphatics in the wall of the stomach takes place from this dense submucosal plexus. Many smaller vessels grow still farther inward and form the scanty plexus adjacent to the muscularis mucosæ. This plexus lies at the base of the gastric glands and sends branches between them.

Figure 8, plate 3, is from a complete injection of the stomach of a pig measuring 150 mm. It has been cut along the greater curvature and spread out to show especially well the marked esophageal ring of lymphatics. This ring constitutes

one of the most striking characteristics of all injections of the stomach made before the appearance of glands in this region. Its formation by vessels arising from the retroperitoneal sac is well shown; branches may be observed passing in every direction from all points of it; those going to the cardiac pouch are well seen over the anterior wall, while in the depths on the posterior wall are seen the vessels of the left gastric trunk with which they anastomose. Those vessels passing to the anterior wall are shown as a series of parallel trunks in the serosa which lead into the deeper, much more extensive plexus of the submucosa within the depths of the stomach wall. These serosal trunks, as well as the plexus of the submucosa, anastomose with the corresponding trunks and plexus arising from the vessels of the greater curvature; they remind one of similar parallel trunks shown by Heuer in the development of the lymphatics of the intestine. The posterior surface of the stomach is seen on the left side of the figure. Here the posterior position of the pyloric vessels arising from the lesser curvature is well shown, as is also the characteristic arrangement of the serosal trunks and plexuses of the submucosa.

The development of lymphatics of the diaphragm is likewise well illustrated by these injections. Large lymphatic trunks, accompanied by the lymphatics of the lower lobes of the lungs which arise from this source, can be seen passing from the anterior part of the retroperitoneal sac to the pillars of the diaphragm. The pulmonary vessels penetrate the diaphragm, while the others spread out over its surface. Also from the gastric plexus, at the lesser curvature of the stomach, many large vessels pass directly to the diaphragm along the ligaments of the liver and anastomose freely with those from the retroperitoneal sac, arriving by way of the pillars (fig. 6). Such an invasion of the diaphragm is readily explained by the double origin of this structure, the pillars arising from the pleuro-peritoneal membrane, while the body of the diaphragm arises from the ventral mesentery, septum transversum, and body-wall. The embryonic lymphatics of the diaphragm are enormous and soon cover the entire structure with a dense plexus. The active function of the diaphragm as an agent of absorption is at once apparent by a glance at its rich supply of lymphatics.

Here a word may be said about the lymphatics of the esophagus and their relation to those of the stomach. Its vessels are derived from two sources. Those of the lower end arise from the gastric vessels forming the periesophageal ring at the cardia of the stomach, from which they spring as direct outgrowths and extend upward to a point on the esophagus about midway between the bifurcation of the bronchi and the cardia of the stomach. Here they meet lymphatics which have extended to the esophagus from the bronchial plexuses at the hilum of the lungs, primarily arising from the thoracic duct. The lymphatic plexuses of the esophagus are completed by the anastomosis of the vessels from these two sources. In their general arrangement they are similar to those of the stomach wall, with which they are continuous. Lymphatic drainage from the esophagus takes place in two directions, the lower portion following the general path for gastric drainage described for the cardia of the stomach, while the upper portion drains to the bronchial plexuses.

Information thus obtained from the injection of embryos did not necessarily justify conclusions in regard to the lymphatic drainage in the fully developed and adult stomach. Injections were therefore made of the stomachs of kittens, cats, and adult pigs. In all specimens thus prepared lymphatic drainage was found to be similar to that deduced from the study of the injected embryos. This was clearly illustrated in the fresh stomach of a kitten 24 days old. Three large glands were seen at the lesser curvature in the gastro-hepatic ligament, one at the base of the splenic ligament, and one lying in the pyloro-duodenal flexure. By injecting directly into the submucosa the paths of drainage were readily seen by the spread of the injection mass through the lympathic plexuses of the stomach wall and its appearance in the adjacent nodes. Injections were made at several sites: (1) By injecting directly into the plexus of the submucosa, in the central region of the anterior and posterior surfaces, the injection mass quickly appears in the nodes of the lesser curvature; also, some of the vessels along the greater curvature are filled by part of the injection flowing in that direction. Thus, the flow from the anterior and posterior walls of the stomach was seen to pass in two principal directions, toward the lesser and toward the greater curvatures, the major part going to the former. (2) Injections near the pylorus and from some distance along the adjacent greater curvature quickly flowed to the gland at the pyloro-duodenal flexure, while, at the same time, an appreciable extent of the anterior and posterior walls and the greater curvature was also filled. (3) When the plexus of the submucosa near the attachment of the gastro-splenic ligament was filled, the vessels passing from the greater curvature toward the spleen, as well as those from the spleen to the large gland at the base of the splenic ligament, were injected. Likewise, from this point of injection, flow occurred freely in all other directions, part going toward the lesser curvature and part toward the pylorus.

Jamieson and Dobson made a careful study of the lymphatic drainage in the fully developed human stomach by means of the method of injection and found the main groups of glands to be associated with the larger arteries of the stomach and spleen. Along the course of the coronary artery they found a group of glands forming a periesophageal ring with another group in the gastro-hepatic ligament. Associated with the splenic artery, in the gastro-splenic ligament, was another group of glands. Along the course of the hepatic artery and in the bend of the duodenum, along the gastro-duodenal artery, still other groups were observed. By injecting directly into the submucous plexus of the stomach wall at various points, the course of the flow of lymph to these glands was studied. From the results so obtained these observers were led to believe that any points of division between areas drained by any definite gland or group of glands were quite arbitrary. They found the plexus of the muscularis to be a very scanty one. The lymphatics of all plexuses of the stomach-wall near the cardia were seen to be continuous with those of the esophagus, whose wall contains lymphatics arranged similarly to those of the stomach. Definite communications were also found between both subserous and submucous plexuses of the pyloric portion of the stomach and the duodenum. These connections of the

subserous plexuses were found to be rather scanty and mostly on the posterior walls of these organs, while the submucous anastomoses were quite numerous.

Such observations are in strict accordance with the ideas one would be led to form from a study of the development of the gastric lymphatics. The three points of invasion from the lesser curvature correspond to the chains of glands along the ascending and descending branches of the coronary artery and the hepatic artery. The lymphatics reaching the stomach by way of the splenic ligaments, however, appear to indicate that lymphatic invasion does not always take place along blood-vessels, since the lymphatics traversing these ligaments are not accompanied by any blood-vessels of appreciable size. More likely, it seems that related folds of peritoneum determine the pathways by which the lymphatics reach the stomach; in many cases the arteries take the same course. The greater number of anastomoses between the vessels of the pylorus and duodenum than has commonly been observed, and the fact that most of the connections between the subserous plexuses of these organs are located on their posterior walls, are points which can be readily understood from the development of the ascending duodenal group of vessels, which are much more numerous in this region (figs. 4 and 6).

So, from the combined evidence offered by injections of developing lymphatics in several embryonic stages, and the further injection of these vessels after the lymphatic plexuses of the stomach have been completely formed, the lymphatic supply of this organ is seen to be one of the richest in the entire body. Due to the homogeneity of its plexuses, no strict division of its areas of drainage can be made that will remain constant in any number of cases. So readily is any portion of the stomach injected from any other part that, in all probability, the lymphatics of the entire organ could be filled by a single injection into the submucosal plexus, if performed slowly enough and with proper pressure. The greatest part of the gastric drainage certainly takes place by way of the lesser curvature, but the other two routes—by way of the splenic ligaments and duodenal vessels—are of quite appreciable capacity. Many factors, such as peristalsis, muscle tone, venous engorgement, pressure in the lymphatic vessels themselves, and (in pathological stomachs) various degrees of obstruction must determine the relative amount of drainage by these three outlets, the sources of whose output are in continuity and show no zonal lines of demarcation.

CONCLUSIONS.

1. From a study of development of the lymphatics in the stomach of the embryo pig, it is found that they invade that organ at two principal points, viz., the lesser curvature, by way of the gastro-hepatic ligament, and the greater curvature, via the ligaments of the spleen.

2. Arising in common with the gastric lymphatics is a large trunk which passes to the duodenum, then up along the duodenum to the pylorus, anastomosing freely with the lymphatics on the posterior surface of the stomach.

3. Part of the lymphatics invading the stomach at the lesser curvature, early in their growth, form a dense periesophageal plexus of vessels in the subserosa.

4. From this periesophageal ring, as well as from the other vessels reaching the stomach at both lesser and greater curvatures, branches are given off at right angles on both the anterior and posterior walls of the stomach. These vessels finally meet and encircle the stomach in a segmental manner similar to that described by Heuer for the intestine. There are a number of connections between these segmental subserous trunks, forming them into a subserous plexus.

5. During the development of this plexus numerous branches from its trunks pierce the layers of the stomach and form other gastric plexuses. All of these are homogeneous layers of lymphatic vessels.

6. The richness of the gastric plexuses and their numerous anastomoses preclude the theory of any sharply defined areas whose drainage would take place in constant given directions.

7. There are four general pathways by which lymphatic drainage may take place: (*a*) the lesser curvature, (*b*) the greater curvature, (*c*) the duodenum, and (*d*) the esophagus.

I wish to thank Professor F. R. Sabin and Dr. R. S. Cunningham for the valuable assistance they have given me in this work, and to express my appreciation to Mr. J. F. Didusch for the illustrations.

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DESCRIPTIONS OF PLATES.

PLATE 1.

FIG. 4. Pig embryo, 60 mm. Anterior view of stomach showing relation of the lymphatics of the lesser curvature to those of the liver and diaphragm. The vessels of the splenic mesentery may be seen extending from beneath the spleen to the greater curvature along which they have grown. Anastomosis has already taken place between these vessels, growing toward the right along the greater curvature, and lymphatics from the lesser curvature, which may be seen coming from the posterior wall of the pylorus. Anastomosis of the ascending duodenal vessels at this point is also shown. (×19.)

A. Duo. V., Ascending duodenal vessels; *D.*, diaphragm; *D. V.*, diaphragmatic vessels; *H.*, liver; *H. V.* hepatic vessels; *S.*, spleen; *V. G. C.*, vessels of the greater curvature; *V. L. C.*, vessels of the lesser curvature; *V. S. M.*, vessels of the splenic mesentery.

FIG. 5. Pig embryo, 40 mm. Posterior view of injected stomach showing the main mass of lymphatics which reach the stomach at the lesser curvature and their growth over the posterior stomach wall. The vessels of the splenic mesentery are also here seen to anastomose with those extending from the lesser curvature. These vessels, which reach the stomach by way of the gastrosplenic ligament, are also shown beginning to extend to the right and left along the greater curvature. The spleen lies in its usual position. The cardiac pouch is well marked in this specimen. (×20.)

C. P., cardiac pouch; *O.*, esophagus; *P.*, pylorus; *S.*, spleen; *V. G. C.*, vessels of the greater curvature; *V. L. C.*, vessels of the lesser curvature; *V. S. M.*, vessels of the splenic mesentery.

PLATE 2.

FIG. 6. Pig embryo, 50 mm. View of stomach from right. The organ has been tilted forward to show the great mass of lymphatics arising from the retroperitoneal sac behind it. The vessels to the duodenum and their extension toward the stomach are shown. Lymphatics from the retroperitoneal sac, some of which ramify over the diaphragm, others piercing it to reach the lungs, are also seen. The thoracic duct lies at the left of the illustration. (×15.)

A. Duo. V., ascending duodenal vessels; *D.*, diaphragm; *Duo. M.*, mesentery of the duodenum; *D. V.*, lymphatics of the diaphragm; *H.*, liver; *H. V.*, hepatic vessels; *L.*, lung; *Pul.. V.*, pulmonary vessels, *R. P. S.*, anterior end of retroperitoneal sac; *T. D.*, thoracic duct; *V. L. C.*, vessels of the lesser curvature.

FIG. 7. Embryo pig, 30 mm. long. Anterior view of specimen from which the liver has been removed but the gastro-hepatic ligament left intact. The dark line, along whose edges are seen cut ends of lymphatics, marks the area from which the liver has been taken. These open vessels are those of the hepatic capsule. The manner in which the lymphatics of the lesser curvature reach the stomach by way of the gastro-hepatic ligament and their relations to the lymphatics of the liver are shown here. The branch of the left gastric trunk passing to the cardiac pouch is also illustrated. The diaphragm may be seen posterior to the cut edge of hepatic peritoneum. (×17.5.)

C. P., cardiac pouch; *D.*, diaphragm; *Duo.*, duodenum; *D. V.*, diaphragmatic vessels; *H. V.*, hepatic vessels; *L. G. T.*, left gastric trunk; *O.*, esophagus; *P. V.*, pyloric vessels; *V. L. C.*, vessels of the lesser curvature.

PLATE 3.

FIG. 8. Embryo pig, 150 mm. long. Dorsal view of stomach which has been cut along its anterior surface parallel to the greater curvature and the specimen spread out to show the relation of the lymphatics of the lesser curvature to those of the anterior and posterior walls of the stomach. The greater curvature is marked by the vessels which have their course along its border (V. G. C.). It may be noticed that the superficial plexus is composed of vessels whose general course is at right angles to the curvatures, while the deep plexus consists in a much denser, homogeneous mass of lymphatics. (×12.)

A. W., anterior wall of stomach; *D. G. P.*, deep gastric plexus (plexus of the submucosa); *O.*, esophageal opening; *P.*, pylorus; *Pan.*, pancreas; *P. W.*, posterior wall of stomach; *R. P. S.*, retroperitoneal sac; *S.*, spleen; *V. G. C.*, vessels of the greater curvature; *V. L. C.*, vessels of the lesser curvature.

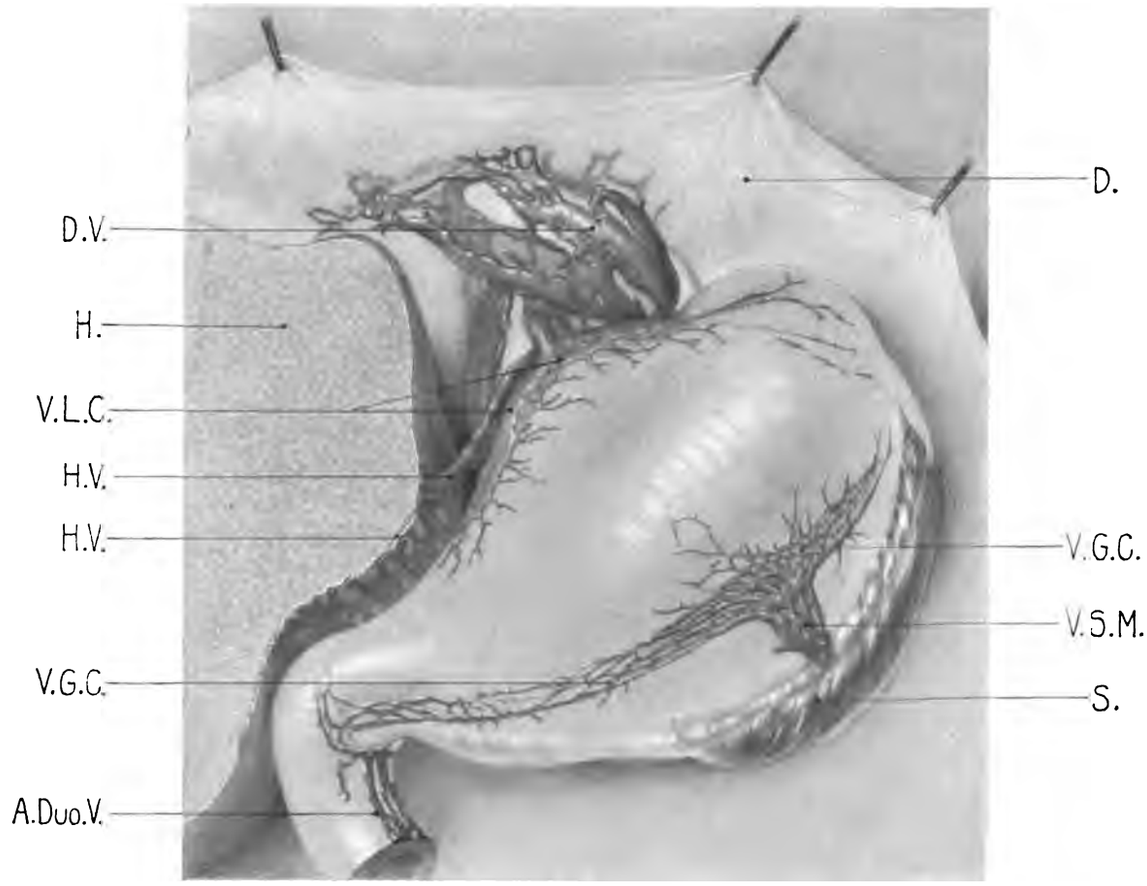

FIG. 4.

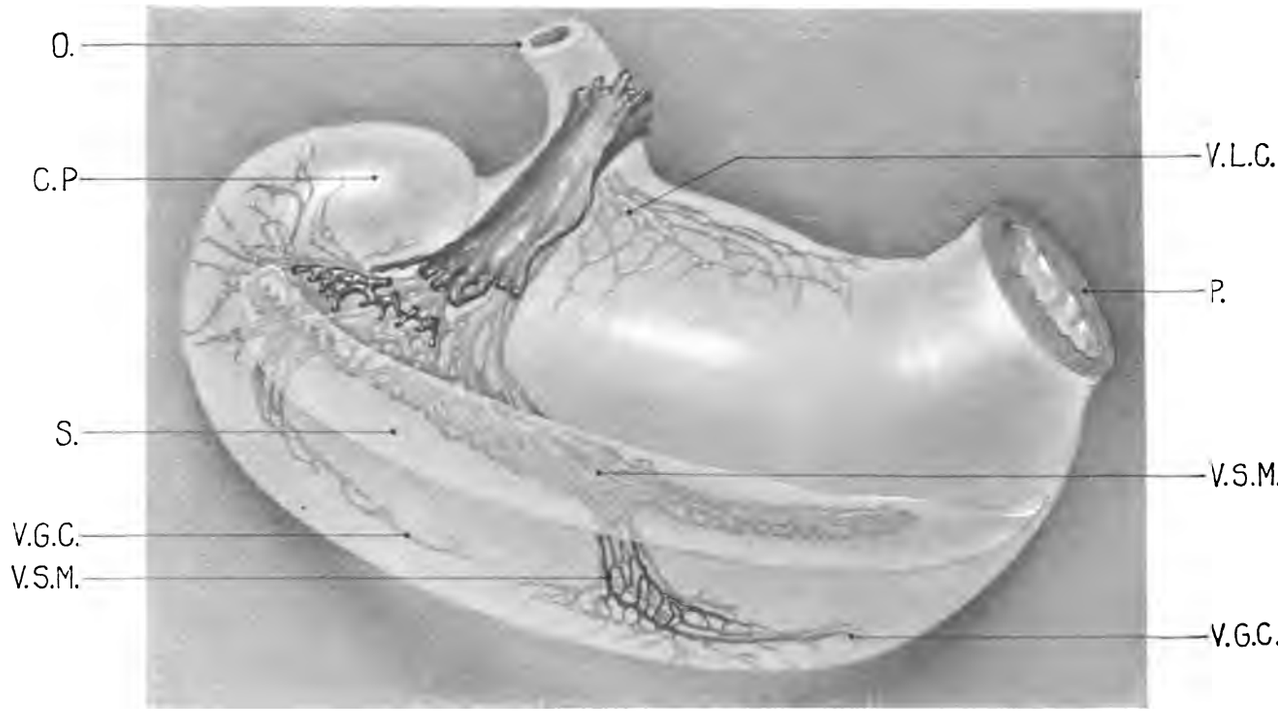

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FIG. 5.

FIG. 6.

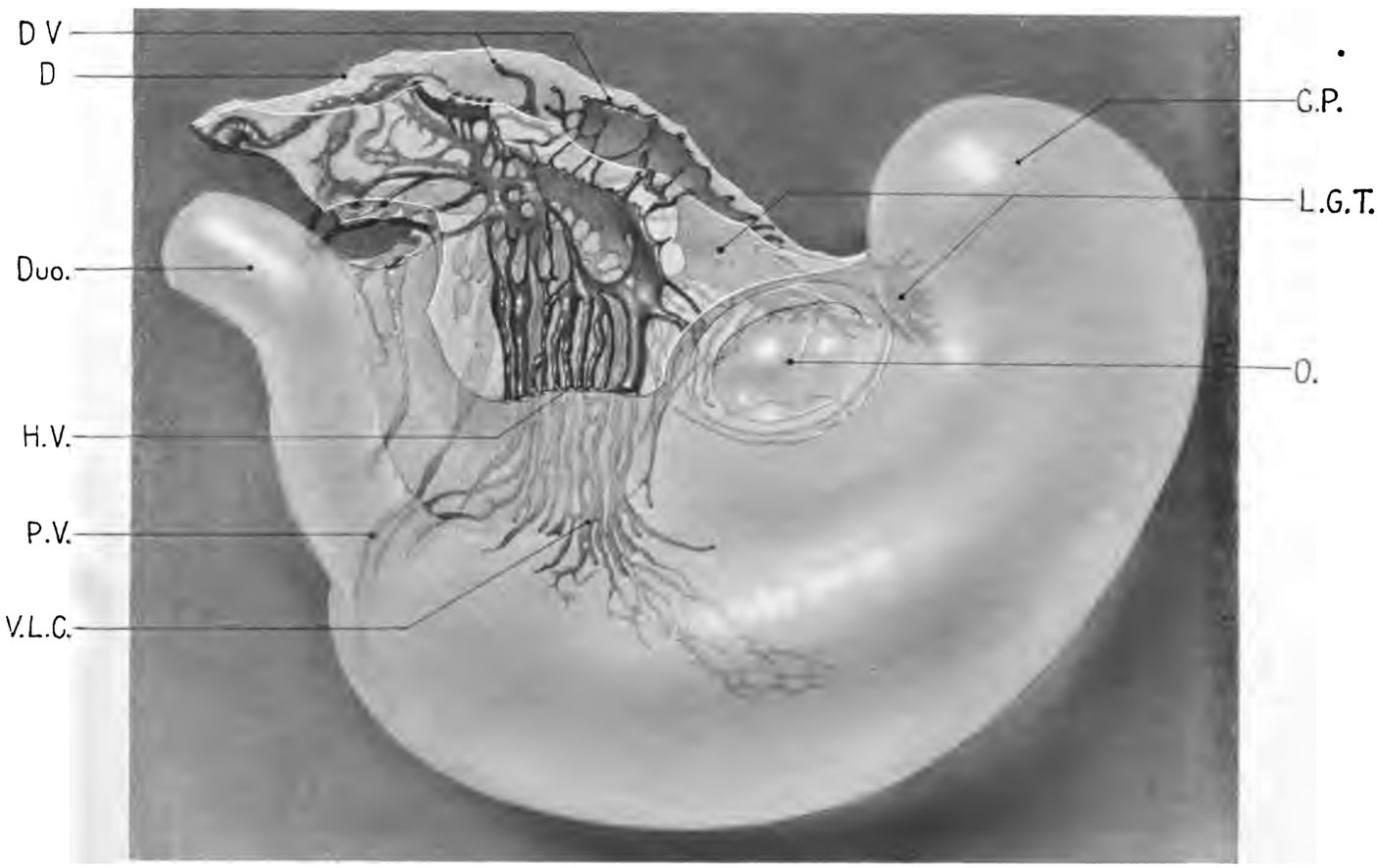

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FIG. 7.

J. R. CASH

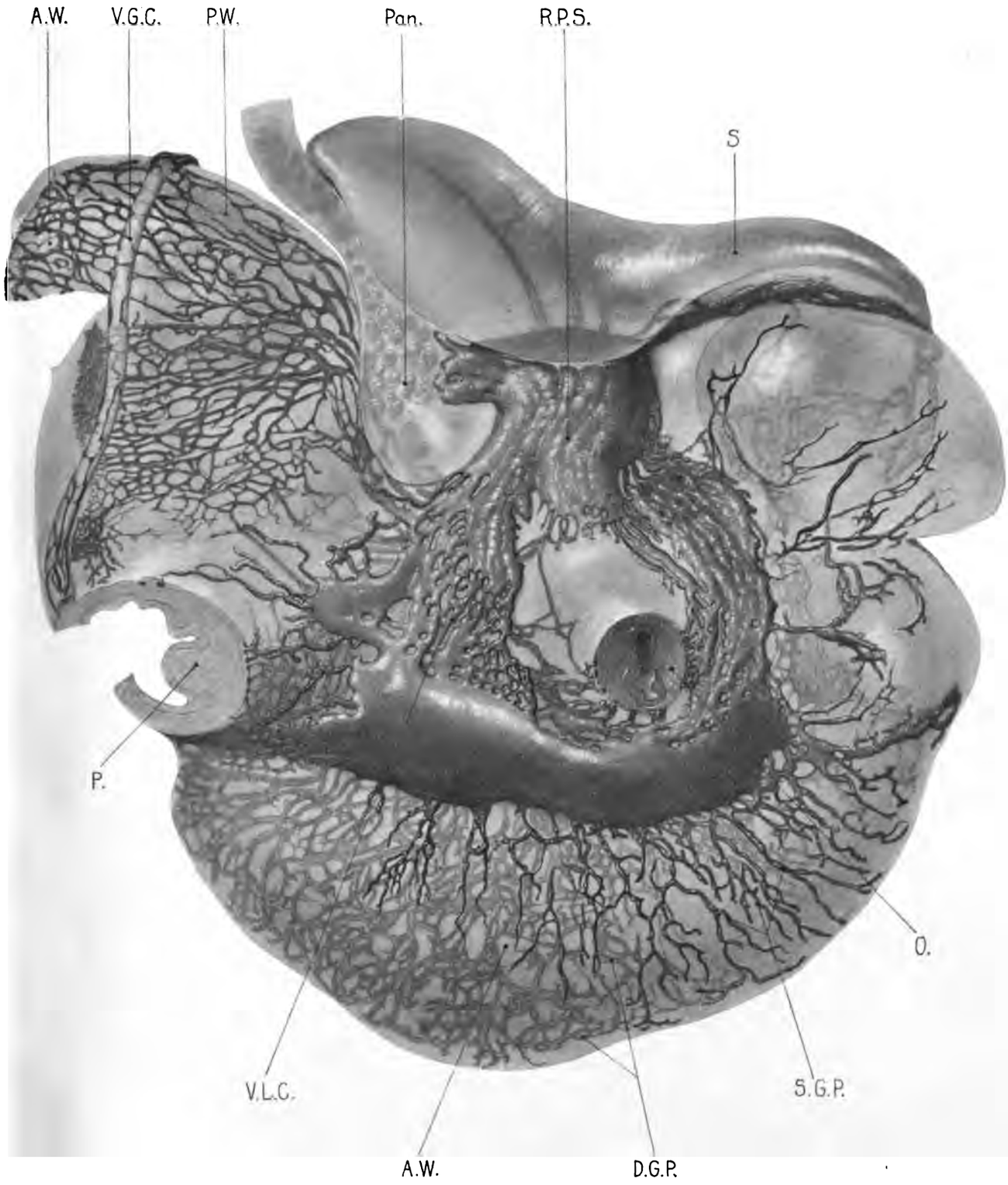

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FIG. 8.

CONTRIBUTIONS TO EMBRYOLOGY, No. 58.

ON THE FATE OF THE PRIMARY LYMPH-SACS IN THE ABDOMINAL REGION OF THE PIG, AND THE DEVELOPMENT OF LYMPH-CHANNELS IN THE ABDOMINAL AND PELVIC REGIONS.

By F. L. Reichert.

With five text-figures.

ON THE FATE OF THE PRIMARY LYMPH-SACS IN THE ABDOMINAL REGION OF THE PIG, AND THE DEVELOPMENT OF LYMPH-CHANNELS IN THE ABDOMINAL AND PELVIC REGIONS.

Since 1900 interest in lymphatics has been centered about the question of their origin and development. The primary lymph-sacs have been worked out and in general their fate determined. Sabin (1901–2) has shown that the earliest lymphatic buds arise in association with the anterior cardinal veins and form the primary lymphatics in the neck—the paired jugular sacs. The other primary sacs originate indirectly from the vena cava through the veins of the Wolffian body. These are the paired iliac sacs, the cisterna chyli (Sabin, 1912) and the retroperitoneal sac (Lewis, 1906; Baetjer, 1908). Quoting from Sabin (1913):

"In most general terms the jugular sacs drain the anterior half of the body; the iliac sacs drain the posterior half of the body; while the retroperitoneal or præ-aortic sac drains the viscera. The cisterna chyli with the thoracic duct connects the jugular and renal lymphatics."

The recent work on the origin of blood-vessels by the differentiation of angioblasts, or vaso-formative cells from mesenchymal cells (Stockard, 1915; Sabin, 1920; Streeter, 1920), may result in modifying our present conception of the origin of the lymphatics to some such form as this: that they arise either directly from the endothelium of the veins or in part by a differentiation of new cells analogous to angioblasts. The experiments of E. R. and E. L. Clark (1920) point in that direction. It is clear, however, that lymphatics begin centrally, close to certain veins, and spread peripherally.

Considering the sequence in the development of the primary lymphatic system, it was at first thought by Sabin (1901) that the earliest lymphatics in the pig were sacs which bud off from certain veins. F. T. Lewis (1906), going back a step farther, showed that in the rabbit the formation of the primary lymph-sacs is preceded by a plexus of blood-filled capillaries connected with the veins. This was confirmed by Sabin (1912) for pig and human embryos, and by Huntington and McClure (1910) for the cat. Recently E. R. and E. L. Clark (1920) have carried the investigation back still farther in the chick, practically to the beginning, when the first evidence of lymphatics consists of a few blood-filled vessels lined with characteristic lymphatic endothelium and connected with veins. These vessels later join to form a circumscribed continuous plexus of vessels which does not have a continuous lumen and which is still connected with the venous system in a number of places. This plexus is the primordium of the lymph-sacs or lymph-hearts, from which the lymphatics of the body arise and grow peripherally. As stated by Sabin (1913), these buds in connection with veins form "a new type of vessels, namely, lymphatics. These buds unite to form plexuses which develop into sacs. These sacs may become lymph-hearts. From these lymph-sacs or hearts lymphatic

capillaries gradually invade the body in orderly sequence in definite and characteristic zones and layers. The growth is always in the capillary bed—that is, all lymphatics develop as capillaries, and the earliest ones to develop become the first lymph trunks or ducts." To make the story more complete (at least in the pig), in the next stage these lymph-sacs break up and are converted into a coarse plexus of lymph-channels, which in turn are transformed into lymph-nodes or glands.

As to the secondary lymphatic system—that is, the peripheral vessels—the development of those of the skin (Sabin, 1904), small intestine (Heuer, 1909), lung (Cunningham, 1916), heart and stomach (Cash, 1917, 1921) has already been studied.

The work of A. H. Clark (1912–13) on the fate of the jugular lymph-sacs gives a general survey of the lymphatics of the anterior region of the body in the pig and shows how the lymphatic channels of the head, neck, and thorax are related to the primary lymph-glands. In the present paper it is desired to give a similar survey of the lymphatics in the abdominal and pelvic regions and to determine more definitely the fate of the primary sacs of the abdominal region. No attempt will be made to present in detail the development of the lymphatics of the different organs.

Throughout the investigation the encouragement and assistance of Dr. Florence R. Sabin have been a constant help, and my sincere appreciation and thanks are due to her.

METHOD.

The material consisted of living pig embryos varying in length from 4 to 20 cm. The lymphatics were injected and the specimens cleared by the Spalteholz method as described by Sabin (1915). Silver nitrate (2 per cent) and india-ink were used for injections. With the latter the injections were nearly complete. Some were made through the retroperitoneal sac, but more thorough injections of the abdominal and pelvic regions were secured by introducing the needle into the thoracic duct on the left side, a short distance posterior to and behind the arch of the aorta, as described by Heuer (1909).

At about the stage of 7 cm., valves are being formed in the lymphatic vessels and the primary sacs no longer remain homogeneous, but begin to break up into plexuses of vessels which are the forerunners of the primary lymph-glands. For this reason, beyond the stage of 8 cm., when it was difficult to get the injection mass to pass the valves, the different organs were injected directly and the lymphatic drainage to the several glands was thus indicated.

In the pig the origin of the lymphatics of the abdominal and pelvic regions can be traced to lymphatics arising ventral to the aorta (fig. 1) and dorso-lateral to the aorta (fig. 4). With the growth of the embryo and the beginning formation of the lymph-glands this distinction can not be made out so readily, since in both cases the glands tend to lie lateral to the aorta. When glands are definitely formed, those arising from lymphatics dorso-lateral to the aorta in the main lie with those from the ventral side.

LYMPHATICS ARISING VENTRAL TO THE AORTA.

RETROPERITONEAL SAC.

In the smaller embryos, beginning at about 23 mm., there is a primary lymphatic sac—the mesenteric (Lewis, 1906) or retroperitoneal sac, as it was termed later (Baetjer, 1908)—situated outside of the peritoneal cavity. This sac lies between the paired Wolffian bodies and gonads, just ventral to the point of renal anastomosis of the subcardinal veins, and extends from the region of the superior mesenteric artery to a point near the bifurcation of the aorta (fig. 1). Up to about 7 to 8 cm. it is a complete sac spreading sheet-like between the Wolffian bodies (Heuer, 1909, figs. 3 and 4), and later between the kidneys.

Lewis (1906) describes the sac in rabbit embryos of 21 mm. as situated at the root of the mesentery, just ventral to the point of renal anastomosis of the subcardinal veins. Baetjer (1908) has shown that it has its origin in numerous venous capillaries which, by increase in number and by fusion, form a sac which has definite connections with the subcardinal veins. There follows a gradual obliteration of these venous connections and for a short period the sac is an isolated structure with irregular margins. By the stage of 25 mm. it has gradually developed connections with the rest of the lymphatic system by an upgrowth of small lymphatic capillaries along the margin of the aorta. Heuer (1909) describes three main connections with the lymphatics dorsal to the aorta:

(1) There are several afferent vessels which arise from the mesial trunk as it leaves the anterior end of the sac and course ventro-dorsad and slightly anterior to enter the thoracic duct (fig. 2, Anas. ant.).

(2) The main efferent connection is a stout trunk (fig. 2, Anas. maj.) opposite the anterior pole of the kidney, between the cisterna chyli and the anterior end of the sac.

(3) A third connection is made from its posterior portion to the caudal part or fan of the iliac sacs. Also, along the renal portion of the aorta there are numerous anastomoses with the lymphatics dorso-lateral to the aorta.

Anterior portion of sac.—This large sac ventral to the aorta may be divided in the region of the hilum of the kidney into an anterior and a posterior portion (fig. 1). The anterior portion gives rise to two main trunks, the large anterior or cœliac trunk and the small posterior or superior mesenteric trunk. Figure 1 shows the ventral or retroperitoneal sac in a pig embryo 7.8 cm., after it had begun to split into a plexus of lymphatic vessels, the forerunners of its transformation into lymph-glands. In the pig it is at this stage a very extensive plexus, in contrast with its earlier sac-like form (Heuer's fig. 4), and its main portions are seen to lie on either side of the aorta, where they form a coarse network of channels. It will be noted that the fold of mesentery and the cœliac trunk have been cut and the stomach turned aside to expose the lymphatics from the anterior end of the sac. At this stage the kidneys have developed into large structures and the Wolffian bodies have begun to degenerate and are pushed to the side. The artist has clearly shown the relationship of the various parts of the reproductive system.

The large cœliac trunk (figs. 1 and 2, T. coel.) immediately divides into a right and a left branch, the latter soon breaking into the mesial trunk and the left trunk. The left trunk, which includes the gastro-splenic trunk, is not shown in figure 1, being on the other side of the fold of mesentery, but it can be well seen in figure 3. The mass of lymphatics at the anterior end of the retroperitoneal sac courses cephalad for a short distance and then turns ventrally, breaking up into several main lymphatic branches. This ventral curve is shown in figure 2. A little way beyond the origin of the mesial trunk, lymphatic vessels pass laterally to spread (at

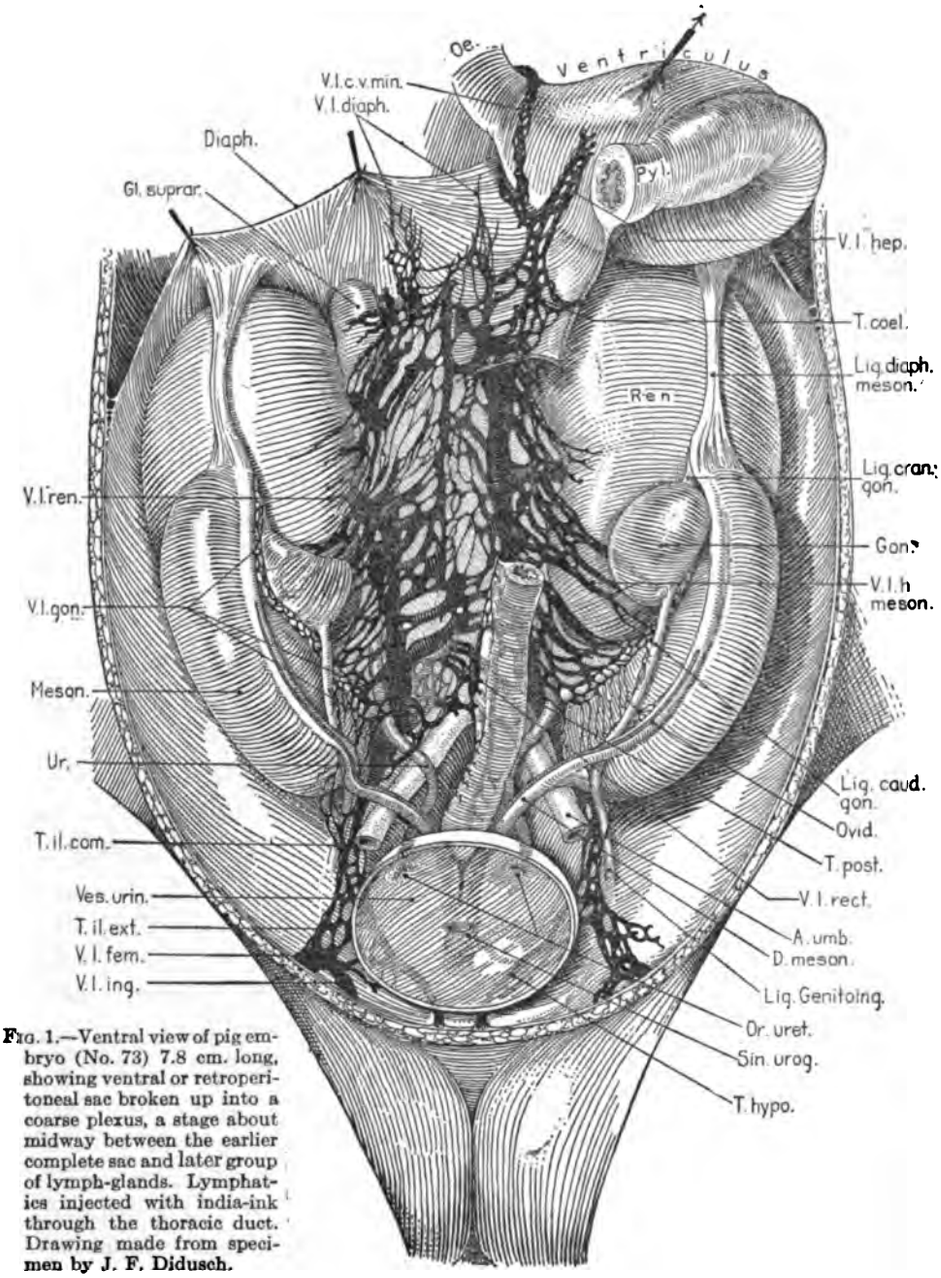

FIG. 1.—Ventral view of pig embryo (No. 73) 7.8 cm. long, showing ventral or retroperitoneal sac broken up into a coarse plexus, a stage about midway between the earlier complete sac and later group of lymph-glands. Lymphatics injected with india-ink through the thoracic duct. Drawing made from specimen by J. F. Didusch.

first in the form of a film, but in older embryos as distinct vessels) over the adrenal capsule and the anterior pole of the kidney (fig. 3, V. l. cap. gl. suprar.). As the trunk twines ventrally around the body of the pancreas, lymphatics are given off to the middle portion of that organ. These pancreatic vessels are not shown in figure 3, but would come off in front of the lower portion of the trunk of the lesser curvature (T. c. V. min.). Laterally, vessels pass from the mesial trunk to the diaphragm (fig. 1, V. l. diaph.), uniting with those from the anterior end of the iliac sacs.

From the mesial trunk, as previously stated, there are several efferent vessels emptying into the thoracic duct (fig. 2, Anas. ant.) On either side of these vessels several long lymphatic channels run cephalad with the pulmonary ligament to supply the lower portions of the lung (fig. 2, V. l. pulm.). The largest trunk of the

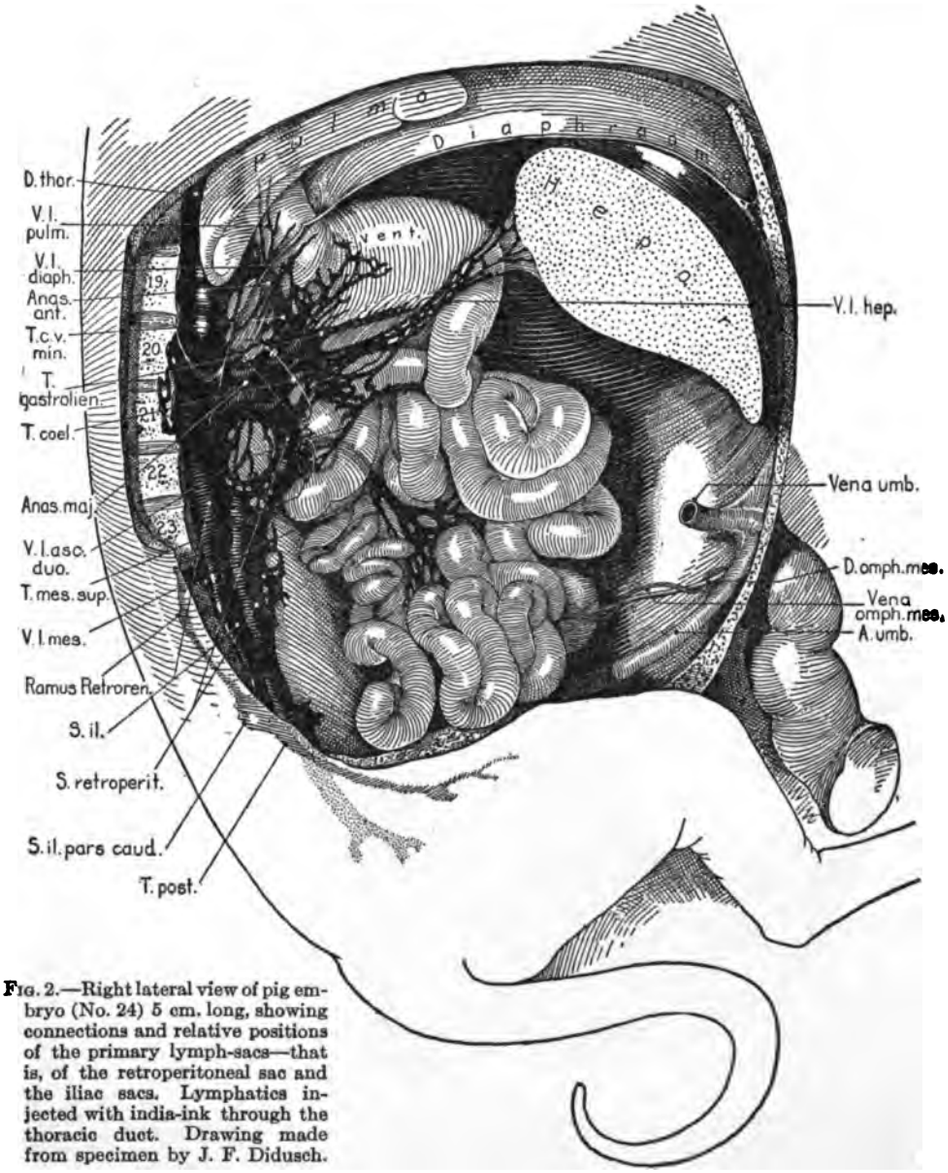

FIG. 2.—Right lateral view of pig embryo (No. 24) 5 cm. long, showing connections and relative positions of the primary lymph-sacs—that is, of the retroperitoneal sac and the iliac sacs. Lymphatics injected with india-ink through the thoracic duct. Drawing made from specimen by J. F. Didusch.

mesial mass is the gastric trunk of the lesser curvature, which is described by Cash (1921). This is shown in part in figure 1, but still better in figure 3, which is a view of the stomach from below, designed to show the main mass of lymphatics of the lesser and greater curvatures and special lymphatics of the pylorus. It shows also the relation of the lymphatics to the pancreas. It is to be noted that the spleen, with the lymphatic vessels in its capsule and mesentery, has been removed and that the gastro-splenic trunk has been cut just as it is dividing into its two terminal branches. The intestines have been removed, leaving only the stump of the superior mesenteric trunk. This gastric trunk terminates at the lesser curvature by dividing into two branches: (1) an ascending or left branch, which supplies the cardiac region of the stomach, forms a network of periesophageal lymphatics, and sends out vessels to the medial portion of the diaphragm; (2) a descending or right branch, which likewise supplies the cardiac region, diaphragm and lesser curvature (fig. 2, V. l. diaph.; T. c. v. min.), and anastomoses with other vessels supplying the diaphragm and lesser curvature.

The left trunk, of which the main branch is the gastro-splenic trunk (fig. 3), passes ventrally to the fundus of the stomach. Near its origin it sends lymphatics to the tail of the pancreas. It continues on to the fundus as the gastro-splenic trunk, then divides beneath the posterior third of the spleen, one branch im-

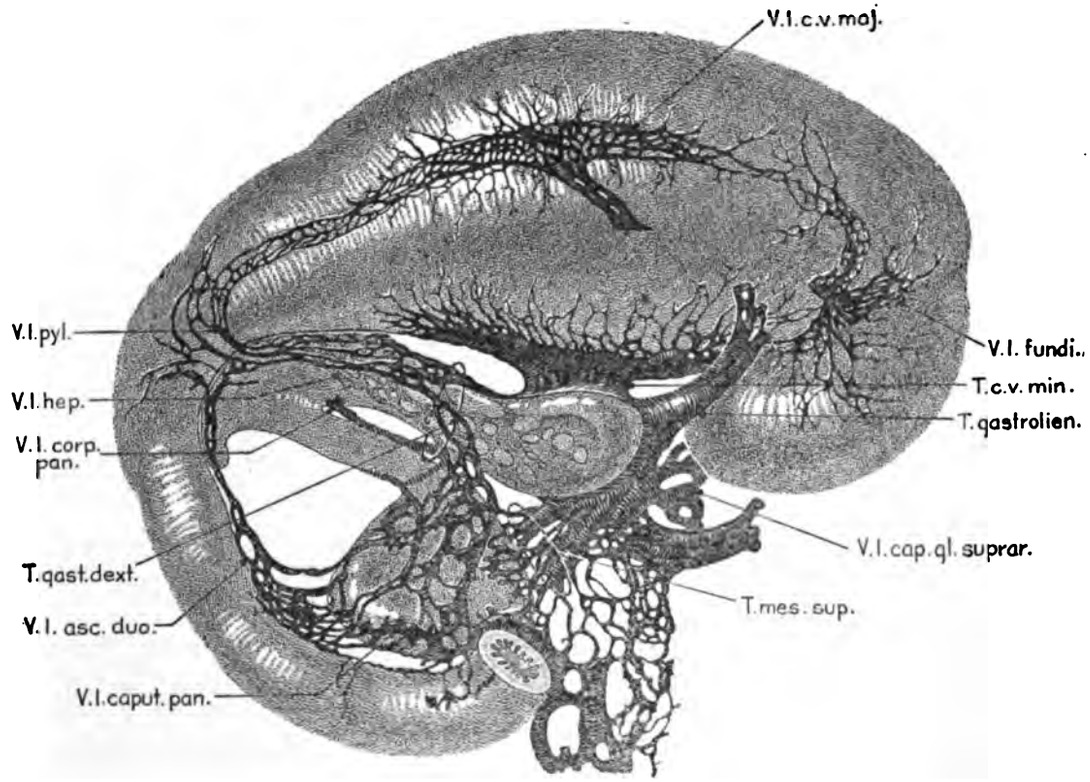

FIG. 3.—Pig embryo (No. 74), 6 cm. long. Ventral view of anterior end of retroperitoneal sac, showing the main gastric trunks and the lymphatics of the pancreas. The spleen has been removed. Lymphatics injected with india-ink through the thoracic duct. Drawing made from specimen by J. F. Didusch.

mediately breaking up to supply the region of the fundus, the other coursing along the length of the spleen in its ligament to a point near the pyloric extremity. Here it again divides, the smaller branch to continue to the pole of the spleen, the larger turning obliquely toward the greater curvature of the stomach, where it forms a T-shaped termination (fig. 3, V. l. c. v. maj.) One limb passes toward the cardiac pouch, breaking into many small vessels on the curvature and finally anastomosing with the vessels supplying the fundus; the other limb passes toward the pylorus and likewise drains the greater curvature and anastomoses with the lymphatics of the pylorus. These anastomoses are well shown in figure 3. In none of the specimens could an injection be secured which involved the parenchyma of the spleen itself.

The right branch arises from the right half of the cœliac trunk and courses ventro-laterally along the gastro-pancreatic line with the hepatic artery, breaking into two branches—the hepatic vessels and the right gastric trunk (fig. 3, T. gast. dex.). From the right gastric trunk the pyloric vessels (fig. 3, V. l. pyl.) pass along the pancreas to the pyloro-duodenal junction, where they turn abruptly forward over the pylorus to anastomose with the lymphatics of the greater curvature. This trunk also sends lymphatics to the body of the pancreas (fig. 3, V. l. corp. pan.).

There are two sets of lymphatics going to the liver. The main mass passes along with the hepatic artery, going behind the pyloric end of the stomach (figs. 1, 2, and 3; V. l. hep.). These vessels unite with the other lymphatics accompanying the biliary ducts to form the hepatic trunk (fig. 2, V. l. hep.). These lymphatics course along with the blood-vessels within the liver in the perivascular and perilobular tissue, but not to the lobule of the organ itself (Mall, 1901). The second hepatic set or trunk arises just posterior to the large anterior or cœliac trunk. This lateral trunk splits off laterally from the right side of the retroperitoneal sac toward the portal vein, which it accompanies for a short distance, then branches at right angles, sending vessels in both directions along that vein. Those passing posteriorly unite with those going to the head of the pancreas. As they approach the liver, these vessels along the portal vein join with those of the hepatic branch from the cœliac trunk to enter at the hilum of the liver, although some accompany the biliary ducts and go to the gall-bladder.

The other main ventral branch from the anterior end of the retroperitoneal sac is the small posterior or superior mesenteric trunk, the stump of which is shown in figure 3. This trunk arises from the sac at about the origin of the superior mesenteric artery and its branches become the satellites of the branches of that artery in the mesentery (fig. 2, V. l. mes.). The trunk appears in figure 2, which is a view of the lymphatics from the side, and shows quite well the relation of the retroperitoneal sac to the iliac sacs, both as to position and connections. It also brings out the enormous mass of lymphatic vessels arising from the anterior end of the retroperitoneal sac. The trunk divides shortly, the smaller (duodenal) branch (figs. 2 and 3, V. l. asc. duo.) supplying the lower portion of that section of the small intestine and anastomosing with the vessels from above. Lymphatics are also given off from this duodenal branch to the head of the pancreas (fig. 3). The other, the mesen-

teric branch (fig. 2, V. l. mes.), is large and supplies the rest of the small intestine as well as the coils of the large intestine, the ascending, transverse, and descending colon.

Posterior portion of sac.—Arbitrarily, the retroperitoneal sac was divided at the hilum of the kidney into an anterior and a posterior portion, but even at the anterior pole the sac is seen to broaden so that it occupies the interval between the mesial borders of the two organs. In earlier stages it extends to the hilum of the Wolffian body. In later stages (7 to 8 cm.) vessels from the lateral side of the sac enter the kidney at the hilum in company with other lymphatics from the dorso-lateral side of the aorta (fig. 1, V. l. ren.). In earlier stages, when the Wolffian bodies are large and completely hide the kidneys in a ventral view, the whole posterior lateral edge of the sac sends a sheet of parallel blunt processes, transverse to the axis of the embryo, which extend a short distance on both the dorsal and ventral capsules of the Wolffian bodies, as well as entering the mesial border in company with the blood-vessels of the glomeruli. At the stage shown in figure 1 the sac does not extend to the mesial border of the Wolffian body, but only a short distance over the ventral surface of the kidney, where numerous stout vessels accompany the mesonephric veins. As these veins atrophy the satellitic lymph-vessels disappear. The remnants of the blunt processes are here shown passing obliquely backward to the posterior part of the Wolffian body and leading to a characteristic plexus along the medial ridge of the Wolffian body—the gonadal plexus (fig. 1, V. l. gon.). Thus, of the numerous blunt processes going to the Wolffian body, only those related to the gonads and other reproductive structures persist; the others disappear and this gonadal plexus becomes the genital branch, appearing in the stage shown in figure 1 as a fine plexus arising from the retroperitoneal sac at its lateral border, just cephalad to the bifurcation of the aorta, and passing laterally to the posterior medial end of the Wolffian body. Here part of the vessels turn sharply and course cephalad along the mesial border of that organ forming a fine plexus about the gonadal blood-vessels. This anterior branch continues to the hilum of the gonad which it supplies. The genital branch also forms a network over the caudal end of the Wolffian body, as well as giving off branches to the Müllerian and Wolffian ducts and the gonadal ligament. In the earlier forms the sac sends lymphatics to the medial side of the umbilical cord, where they anastomose with those from the posterior portions of the iliac sacs. In the later stages they fail to inject.

Thus the peripheral spread of the lymphatic vessels from the posterior part of the retroperitoneal sac has been described; it remains only to mention the third efferent connection, which arises from the posterior end of the retroperitoneal sac, passes laterally to the outer side of the umbilicus, and then turns dorsally to join the caudal ends of the iliac sacs.

LYMPHATICS ARISING DORSO-LATERAL TO THE AORTA.

Besides the paired anterior or jugular sacs there is another pair, the iliacs, which grow along the dorso-medial edge of the Wolffian body, dorso-lateral to the aorta, and drain the posterior half of the body. Figure 4 shows these two iliac sacs slightly dorso-lateral to the aorta and also their relation to the cisterna chyli and thoracic duct. It will be noted that there is an extensive plexus between the two sacs, while the paler vessels in the depth are from the retroperitoneal sac. The remnants of the Wolffian bodies show at the posterior borders of the kidneys. The iliacs are connected with the jugulars by the cisterna chyli and thoracic duct. Sabin (1912) has shown that the cisterna chyli and iliac sacs arise from the mesonephric veins of the vena cava.

In the younger specimens, in the region of the diaphragm the thoracic duct is seen as two vessels, one on either side of the aorta, lying dorsally. These anastomose freely in front of and behind the aorta by lateral vessels. At about the same level as the anterior end of the retroperitoneal sac the vessels of the thoracic duct unite to form posteriorly an irregular dilatation, the cisterna chyli, extending to a point near the posterior poles of the kidneys.

From the lymphatics lying dorso-lateral to the whole length of the aorta—that is, from the thoracic ducts, the cisterna chyli and the anastomosing iliac sacs—other lymphatics are given off dorsally as satellites of the segmental arteries which accompany the anterior and posterior rami. No vessels were observed, however, to enter the vertebral canal with the spinal branch of the posterior ramus, which is in accord with previous embryological studies, from which it was evident that the central nervous system contains no lymphatics.

ILIAC SACS.

Caudal to the cisterna chyli, and draining into it, are two large trunks, one on either side of the aorta. These are the paired iliac sacs, which in earlier stages appear to be united. Small at the cephalic end where they form the cisterna chyli, they broaden out gradually to form a rectangular sac between the dorso-medial surfaces of the kidneys and show two expansions. The lateral one is at the hilum of the kidney to which it sends vessels and a small trunk extending laterally in the body-wall to a retrorenal position, then turning abruptly caudad to unite with a trunk from the posterior portion (fig. 2, Ramus retroren.). In older embryos the latter gradually disappears. The other expansion occurs at the posterior end and may be called the caudal portion of the iliac sac. It is roughly fan-shaped, the expansion being latero-posterior, the outer margin curving with the posterior pole of the kidney (fig. 4, S. Il. pars caud.).

In later stages, even at 5 cm., a distinct change is noted in the lymphatics dorso-lateral to the aorta (fig. 4). The iliac sacs are no longer fused, but come to lie on either side of the midline between the aorta and the medial edge of the kidney. They empty through one or two trunks into the cisterna chyli in the region of the adrenals. Their median border, accompanying the aorta, is straight and may sometimes, even in the earlier stages, send anastomosing vessels to the other side.

With the growth of the embryo these sacs come to lie entirely lateral to the aorta in approximation to and above the retroperitoneal sac, which likewise has become situated lateral to the aorta.

These three sacs are connected by small channels which show in figures 2 and 4, and when they are transformed into lymph-glands the result is a rather confusing

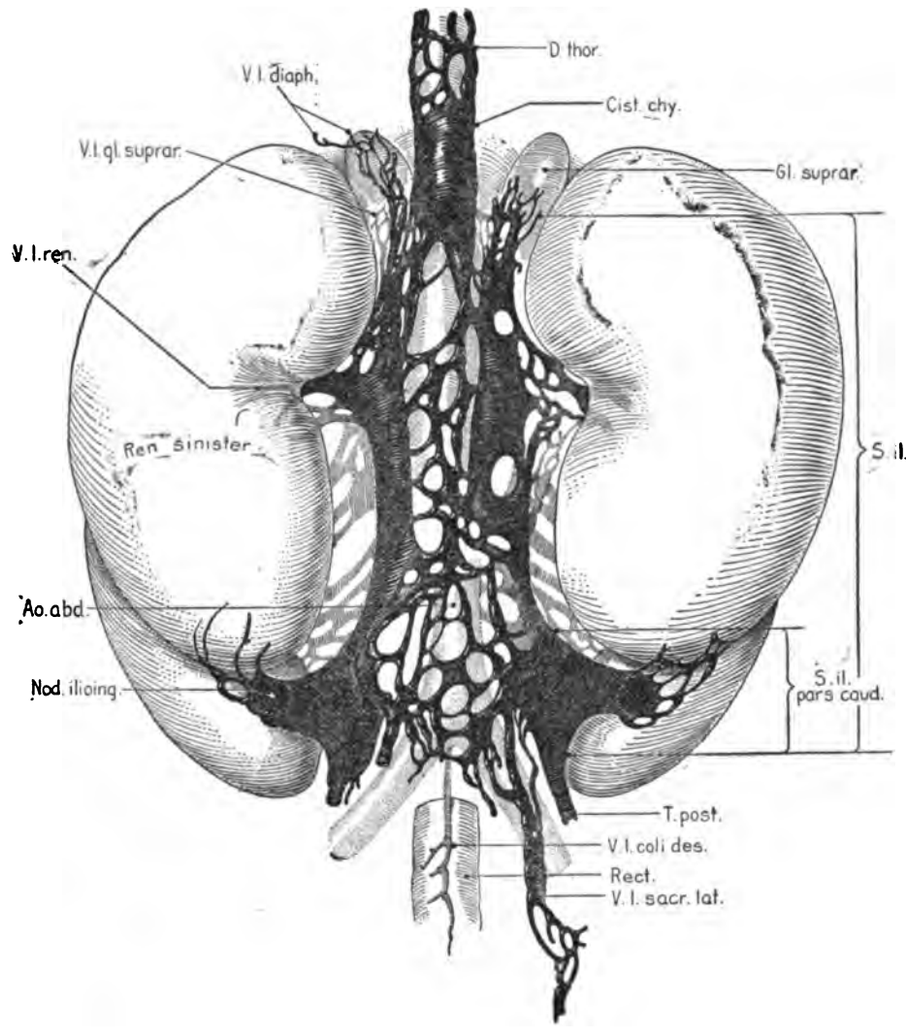

FIG. 4.—Dorsal view of specimen seen in figure 1, showing primary lymphatics dorsal and dorso-lateral to the aorta, namely, the thoracic duct, cisterna chyli, and the paired iliac sacs. Lymphatics injected with india-ink through the thoracic duct. Drawing from specimen by J. F. Didusch.

mass of right and left juxta-aortic lymph-nodes which is made up of glands arising from lymphatics both dorsal and ventral to the aorta, and which extends from just above the hilum of the kidney to the bifurcation of the aorta, forming the main mass of lumbar nodes of the adult. Since the glands arise not only from the ventral and dorsal sacs, but probably also along some of the anastomosing vessels between these sacs, the resulting lymph-glands in the adult are exceedingly

difficult to analyze in terms of dorsal and ventral lymphatics. However, there are glands arising from a small dorsal trunk or plexus which form the post-aortic or prevertebral nodes, but these are few in number (fig. 5, Lymgl. praevert.).

From the cephalic end of the iliac sacs lymphatics pass forward, anastomosing with those from the retroperitoneal sac to supply the diaphragm (fig. 4, V. l. diaph.). The adrenals receive several vessels (V. l. gl. suprar.) which arise from this cephalic end of the sacs in company with those which pass to the diaphragm. As mentioned previously, the kidney receives at its hilum vessels from the iliac sac (fig. 4, V. l. ren.). There appears, therefore, to be a double supply to that organ, the vessels from the iliac sacs and those from the posterior half of the retroperitoneal sac. These vessels do not seem to penetrate very deeply into the kidney. Besides the vessels to the hilum of the kidney, a rather stout trunk, broad at the base, arises at this point and extends laterally in the dorsal wall. At times this is quite a large lateral expansion from the iliacs and forms a retrorenal sac which gives off one or more branches passing posteriorly at right angles to the body-wall, and anastomosing with similar vessels from the caudal end of the iliacs (fig. 2, Ramus retroren.). It also sends a few vessels into the lateral body-wall, where a small plexus is formed which drains part of the deeper layers of the wall. Very few if any superficial vessels drain into it. As stated before, in older embryos these retrorenal branches could not be injected.

Caudal portion of sac.—The fan-shaped caudal portion of each iliac sac lies in the body-wall between the posterior pole of the kidney and the bifurcation of the aorta (fig. 4). It extends laterally and dips ventrally on its mesial side. Later it forms an inverted Y-shaped expansion, one branch passing laterally along the posterior pole of the kidney, the other going directly caudal and then turning ventrally to supply the lower portion of the body and legs.

From the lateral branch, in the earlier stages, a few vessels or a small trunk course cephalad to anastomose with the vessels from the retroperitoneal sac. Laterally, this caudal portion sends a group of lymphatics through the body-wall to form a superficial node just above the crest of the ilium (the ilio-inguinal node) which is characteristic for the pig, and drains the skin of the posterior half of the body and tail (Sabin, 1904). From the medial side of the posterior branch of the caudal portion one or two vessels pass caudally in the dorsal wall along with the lateral sacral vein (fig. 4, V. l. sacr. lat.). On the postero-medial side of the sac a short trunk extends ventrally, medial to the umbilical artery, to anastomose with another trunk from the retroperitoneal sac.

The posterior trunk (fig. 4, T. post.), which drains the lymphatics of the legs and some of the pelvic organs, is the continuation of the medial branch of the caudal portion, which courses ventrally and laterally, passing lateral to the umbilical artery along the common iliac artery (fig. 1, T. il. com.). Only the proximal portion of the posterior trunk is shown in figure 4, for as it turned ventrally it became less and less distinct from the dorsal side of the embryo; but in figure 1, which is a ventral view of the same specimen, the continuation of the posterior trunk is well seen. It divides into several branches, two of which are proximal. From its medial edge, near its origin, the trunk sends a vessel ventro-medially to the lateral side of

the umbilical cord, where it anastomoses with a similar vessel from the retroperitoneal sac. It also sends lymphatics ventrally, in company with the vessel to the umbilical cord, to break up into smaller vessels over the posterior pole of the Wolffian body, where it evidently supplies part of the Müllerian and Wolffian ducts (shown on the left side of fig. 1) just at the inferior pole of the Wolffian body. This seems an important point, for, as will be noticed in figure 1, the lymphatics of the gonad itself are related primarily to *one* set—the ventral lymphatics, derived from those of the Wolffian body—while the lymphatics of the accessory genital organs are shown to be embryonically related to *two* sets of lymphatics—those from the ventral or retroperitoneal sac, and those from the dorso-lateral or iliac sacs.

The posterior trunk then continues as a definite group of lymphatics, the common iliac trunk following the artery of that name. It terminates by dividing into two smaller branches, the more important of which is the femoral or external iliac. These terminal vessels of the posterior trunk are satellites of the main arteries of the pelvis and hind-limb (fig. 1). In the stages at which the injections were made, the first or primitive arrangement of the blood-vessels of the posterior part of the body has changed, so that the blood comes from the aorta by way of the two common iliac vessels, which arise dorso-laterally just anterior to the origin of the umbilical arteries. Each passes caudally, lateral to the umbilical cord, sending a branch (the ilio-inguinal) around the pole of the kidney. At or just beyond the passage into the pelvic wall it divides into two main vessels. One of these, the internal iliac or hypogastric, which runs dorso-medially, divides into a number of terminal branches. The other, the femoral or external iliac, continues ventrally to the front of the hind-limb. At the proximal end of the femoral the now atrophic sciatic artery arises.

The common iliac lymphatic trunk broadens considerably at the bifurcation of the common iliac artery and then divides into a ventral and dorsal branch. The dorsal trunk—the internal iliac or hypogastric (fig. 1, T. hypo.)—accompanies the internal iliac artery and terminates by breaking into a number of vessels, satellites of the lesser arterial branches. A short distance from its origin the ventral branch—the femoral or external iliac (T. il. ext.)—sends a few lateral branches with the sciatic artery. It divides shortly, many of the vessels turning medially to form the inguinal trunk (fig. 1, V. l. ing.) which drains a plexus of lymphatics along the inguinal ligament. This plexus is converted into two large elongated nodes, one situated more deeply than the other. These break up into a group of deep and superficial nodes which drain the lower abdominal wall, external genitalia, perineum, mesial side of the thigh, and practically the whole of the lower extremity. The remainder of the vessels of the femoral trunk (fig. 1, V. l. fem.) continue caudally, following the femoral artery in its course in the leg.

Relation of the Peripheral Lymphatics to the Primary System.

I. Lymphatics arising *ventral* to the aorta.
- A. Retroperitoneal sac.
  - 1. Anterior portion.
    - (*a*) Cœliac trunk.
      - (I) Left branch.
        - (A) Mesial trunk.
          - (1) Adrenal (capsule) and kidney (ant. pole).
          - (2) Pancreas (body).
          - (3) Diaphragm.
          - (4) Anterior anastomosis to thoracic duct.
          - (5) Lung.
          - (6) Trunk of lesser gastric curvature.
            - (*a*) ascending (left) periesophagus, cardia, diaphragm.
            - (*b*) descending (right) cardia, diaphragm, lesser curvature.
        - (B) Left trunk.
          - (1) Pancreas (tail).
          - (2) Gastro-splenic trunk.
            - (*a*) Fundus of stomach.
            - (*b*) Splenic capsule.
            - (*c*) Vessels of greater gastric curvature; (1) fundus; (2) pylorus.
      - (II) Right branch.
        - (A) Hepatic vessels.
        - (B) Right gastric vessels; (1) pylorus; (2) pancreas (body).
    - (*b*) Lateral trunk.
      - (I) Portal vein.
      - (II) Biliary ducts.
    - (*c*) Superior mesenteric trunk.
      - (I) Duodenal branch; (A) pancreas (head).
      - (II) Mesenteric branch.
    - (*d*) Main anastomosis to cisterna chyli.
  - 2. Posterior portion.
    - (*a*) Lateral branches.
      - (I) Kidney.
      - (II) Wolffian body.
      - (III) Genital branch.
        - (A) Gonad.
        - (B) Wolffian body (caudal).
        - (C) Müllerian and Wolffian ducts.
        - (D) Caudal gonadal ligament.
      - (IV) Umbilical cord (medial side).
      - (V) Posterior anastomosis to caudal end of iliac sacs.
    - (*b*) Medial branch.
      - (I) Descending colon.

II. Lymphatics arising *dorso-lateral* to the aorta.
- A. Thoracic duct.
- B. Cisterna chyli.
- C. Iliac sacs.
  - 1. Diaphragm.
  - 2. Adrenal.
  - 3. Kidney.
  - 4. Retrorenal branch.
  - 5. Caudal portion.
    - (*a*) Anastomosis with retrorenal branch.
    - (*b*) Ilio-inguinal node.
    - (*c*) Lateral sacral branch.
    - (*d*) Posterior anastomosis with retroperitoneal sac.
    - (*e*) Posterior trunk.
      - (I) Proximal branches.
        - (A) Umbilical cord (lateral side).
        - (B) Wolffian body (posterior pole). (1) Müllerian and Wolffian ducts.
        - (C) Rectum.
      - (II) Common iliac trunk.
        - (A) Hypogastric trunk.
        - (B) External iliac trunk; (1) sciatic vessels; (2) femoral vessels; (3) inguinal vessels.

The schema given on the preceding page shows graphically the relation of the peripheral lymph-vessels to the primary lymphatic system. In general the lymphatics arising ventral to the aorta (the retroperitoneal sac) drain the whole or part of the lung, diaphragm, liver and biliary passages, stomach, small and large intestine, capsule of spleen, pancreas, kidney, gonad, Müllerian and Wolffian ducts, umbilical cord, and Wolffian body.

From the lymphatics arising dorso-lateral to the aorta (the iliac sacs) are supplied, either wholly or in part, the diaphragm, body-wall, adrenals, kidney, bladder, umbilical cord, Müllerian and Wolffian ducts, and the entire posterior half of the body.

The structures which drain into the primary lymphatics arising both ventral and dorso-lateral to the aorta are the diaphragm, kidney, and Müllerian and Wolffian ducts.

RELATION OF THE LYMPH-GLANDS TO THE PRIMARY AND SECONDARY LYMPHATIC SYSTEMS.

Sabin (1913) has shown that the primary lymph-nodes develop from the primary lymph-sacs; that the pre-aortic or retroperitoneal glands develop from the retroperitoneal sac; and that from the iliac sacs there arises a chain of small nodes lateral to the aorta and a large group of glands on either side of the aorta opposite its bifurcation. Dorsal to the aorta are the prevertebral nodes extending from the lower end of the cisterna chyli to the aortic bifurcation.

Of the secondary lymphatic nodes developing along the lymphatic vessels, she states that the mesenteric glands are secondary for the retroperitoneal sac. In the pig the secondary glands from the iliac sacs are simple and two in number—the ilio-inguinal gland (very characteristic for this animal) and the inguinal group of glands.

In this study, injections, as nearly complete as possible, were made of pig embryos 20 cm. or more in length to determine what glands receive the drainage from the abdominal and pelvic viscera. At this stage the glands are easily seen in the gross and, by injecting the organs with india-ink, their relations to the different glands can be readily demonstrated. Figure 5 is a composite drawing of an embryo pig 20 cm. long, viewed from the ventral side, to show the grouping and positions of the various lymph-glands relative to each other and to the different abdominal organs. Those glands formed from lymphatics arising ventral to the aorta are indicated in solid color; those from lymphatics dorso-lateral to the aorta are stippled. The glands along the vessels posterior to the bifurcation of the aorta, however, which are in solid black, belong to the group arising from lymphatics dorso-lateral to the aorta and were not stippled because of the perspective.

In the abdomen are glands—the pre-aortic—extending from above the cœliac axis to the bifurcation of the aorta. Anteriorly these are grouped into the cœliac (fig. 5. Lymgl. cœl.) and main mesenteric glands (Lymgl. mes. sup.) and posteriorly into the lumbar pre-aortics (Lymgl. praeaorticæ). Lateral to the aorta are the

juxta-aortic glands; on the right side some are prevenous, others retrovenous, in relation to the vena cava (fig. 5). Posterior to the aorta are the prevertebral glands. Glands are formed along the external iliac artery and smaller ones about the internal iliac or hypogastric artery.

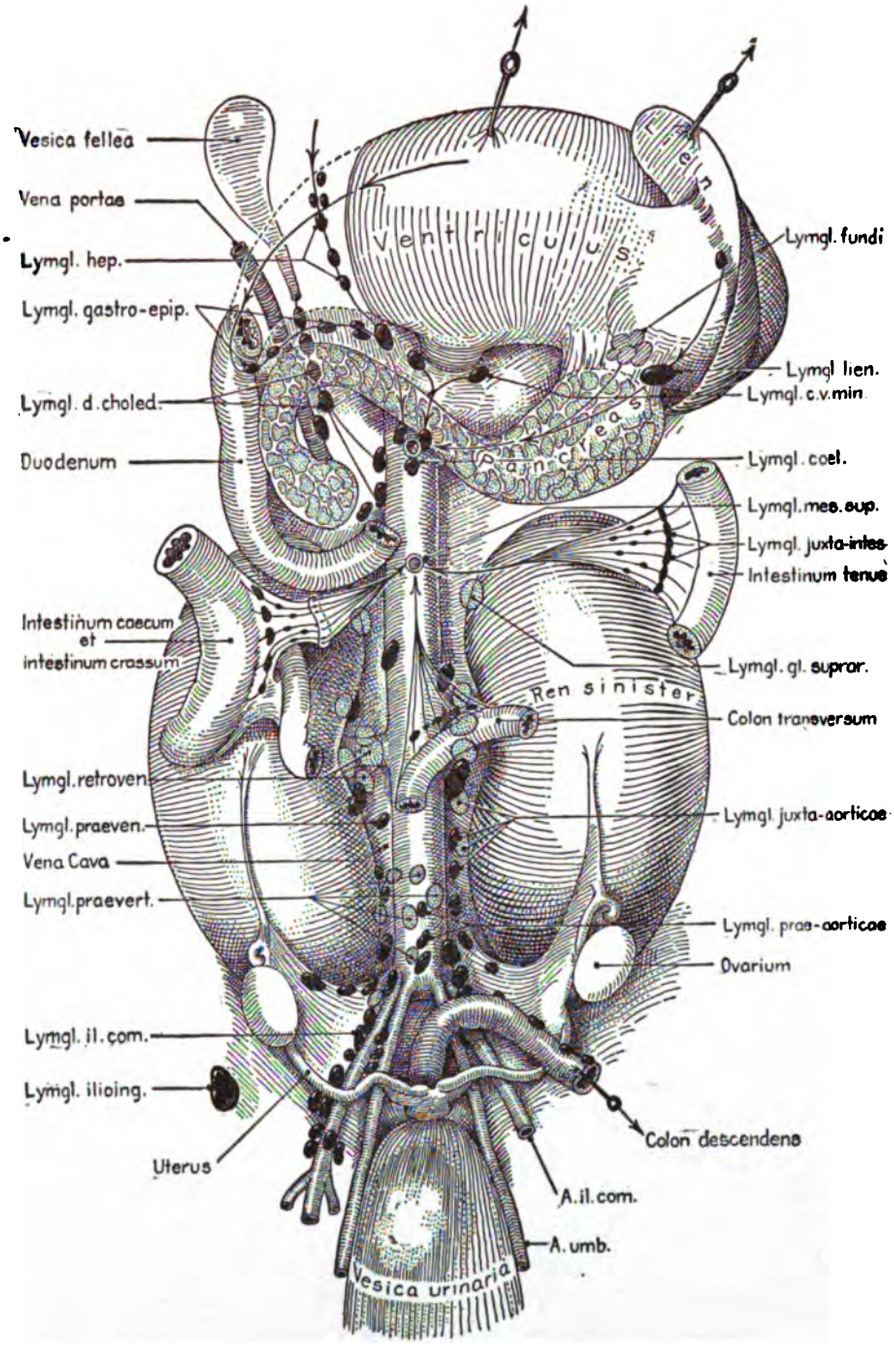

FIG. 5.—Composite diagrammatic drawing viewed from the ventral side of a pig embryo 20 cm. long. The glands arising from the retroperitoneal sac are in solid color, those arising from the iliac sacs are stippled. However, the glands along the vessels posterior to the bifurcation of the aorta, which are in solid black, belong to the group arising dorsolateral to the aorta, but were not stippled because of the perspective. Drawing made by J. F. Didusch.

The retroperitoneal sac is transformed into the glands of the cœliac axis and, at the origin of the superior mesenteric artery, into the main mesenteric glands and also into the preaortics, the prevenous, and part of the juxta-aortic glands. The iliac sacs become the retrovenous, prevertebral, and the remainder of the juxtaaortic glands. The glands constituting the large group opposite the bifurcation of the aorta, as well as the proximal iliac glands, are formed from the caudal ends of the sacs.

DRAINAGE OF THE VISCERA.

It has been shown that in the earlier stages the stomach has three main systems of drainage: (1) the trunk of the lesser gastric curvature, (2) the gastrolineal trunk, and (3) the right gastric branch. This system is likewise indicated by the lymph-glands, the three main channels of drainage being indicated by arrows in figure 5.

1. In a pig of 20 cm., when the injection is made about the periesophageal region, the vessels shown on the anterior side of the fundus and about the cardiac orifice drain around the neck and then posteriorly to several large nodes situated slightly posterior and to the left of the orifice on the pillar of the diaphragm (fig. 5, Lymgl. fundi.). Other lymphatic vessels, more from the middle portion of the stomach, drain to a gland on the right of the cardiac orifice (Lymgl. c. v. min.).

2. Lymph from the inferior part of the fundus drains along the posterior margin of the splenic ligament, following the lesser gastric vessels of the lienal artery, where it empties into several glands lying in the splenic ligament. From these nodes it drains posteriorly into several large glands near the left periesophageal glands (fig. 5, Lymgl. fundi). Part of the lymph from the greater curvature drains with that from the gastro-epiploic vessels to the middle region of the spleen, where it enters several glands. Connection is demonstrated between these glands and nodes situated along the splenic vessel. Not only these glands, but also the periesophageals, follow the splenic artery to the cœliac axis (fig. 5, Lymgl. cœl.). From the superior and inferior medial regions of the stomach, vessels run toward the lesser curvature, along with the branches of the left gastric artery, to drain with a number of smaller nodes which are located to the left of the esophageal ring (fig. 5, Lymgl. c. v. min.) and drain, along with the right periesophageals, to the glands about the cœliac axis.

3. Lymphatics injected on the superior surface of the stomach near the pylorus drain in the direction of the lesser curvature, but run in the lesser omentum, turning with the vessels and emptying into very large glands situated along the hepatic artery (fig. 5, Lymgl. hep.). These hepatic glands also have as afferents the vessels of the greater curvature; that is, the lymphatics of the greater curvature pass toward the pylorus accompanying the right gastro-epiploic artery (fig. 5, Lymgl. gastroepip.), following that vessel from its junction with the gastro-duodenal to the hepatic artery, where they drain into these large nodes. Thus, about the cœliac axis are a number of glands that in the earlier stages were in the form of trunks from the anterior end of the retroperitoneal sac, and they, too, show three main systems of drainage—a left, a mesial, and a right.

In the lesser omentum are small lymph-glands draining part of the liver. The drainage from these nodes is seen to accompany the vessels from the pylorus which

follow the right gastric artery to empty also into the large hepatic glands, and from these into the cœliac glands. When the gall-bladder was injected, some vessels were seen to follow the course of the common duct to the duodenum, then to accompany the portal vein to one of several large nodes situated about that vessel in the angle of the pancreas (fig. 5, Lymgl. d. choled.). Smaller glands were also seen along this vein peripheral to the larger ones. The efferents from these glands go to one or two pre-aortic nodes lying between the cœliac axis and the superior mesenteric nodes (fig. 5). Here, too, as in the earlier stages, there is a double drainage of the liver.

In these later stages the lymphatics of the spleen failed to inject. Those of the ligament, however, drain into glands at the hilum, from which the efferent flow is through glands which are disposed about the splenic artery.

The small intestine, large intestine, and descending colon drain into the large mesenteric glands, of which there are several groups. The largest and most important of these are the juxta-intestinal glands (fig. 5, lymgl. juxta-intes.) which are the first to receive the lymph coming from the intestine. They are very large prominent, elongated glands, lying close together, their long axes parallel to that of the intestine. They form a distinct nodular rim or collar to the outer part of the mesentery. Efferents from the juxta-intestinal nodes pass through intermediate glands in the mesentery about the origin of the superior mesenteric artery (Heuer, 1909). These retromesenteric glands then receive afferents from the head of the pancreas, duodenum, and intestine. This system of secondary lymph-glands of the intestine, with the peripheral nodes very large and prominent, and the proximal ones smaller and very limited in number, is quite a contrast to the system of the stomach and neighboring organs, in which the glands increase in size and importance the nearer they approach to their termination about the cœliac axis.

The lymphatics of the pancreas could not be injected directly, but there are indications that this organ has at least four collecting trunks: (1) The drainage from its tail is to the splenic group of glands; (2) the posterior part of the body sends some efferents to the periesophageal nodes; (3) the duodenal portion drains along with the lymph from the pyloric portion of the stomach to the large hepatic nodes; (4) the head of the pancreas sends efferents to the superior mesenteric glands. Bartels (1906) was able to inject the lymphatics of the pancreas in newborn animals, especially the dog, and in a later paper (1907) he described their drainage into the regional glands, which, in general, corresponds to the drainage indicated above.

It is evident from the earlier stages that the adrenals have a capsular drainage to the cœliac group of glands, while the lymph from the organ itself drains into a gland formed from the cephalic end of the iliac sac, *i. e.*, a juxta-aortic gland which is seen lying anterior to the renal glands (fig. 5, Lymgl. g. suprar.).

Although not definitely determined, it would seem from the double type of earlier injections that the lymphatics from the kidney would drain into the pre-aortic and perhaps post-aortic, but mainly into the juxta-aortic glands. Several large nodes can be easily seen at the hilum lying behind the renal vessels, suggesting

their origin from the iliac sacs, and a fairly large node anterior to the vessels suggests its origin from the retroperitoneal sac. The separation of the juxta-aortic glands by the vena cava, into a prevenous and a retrovenous group which were formed from lymph-sacs ventral and dorso-lateral to the aorta, is shown in the right side in figure 5, where the double drainage from the kidney to these two sets of glands at the hilum is indicated.

In young embryos, vessels from the region of the inferior mesenteric artery pass to the colon, and in later stages glands are seen about the inferior mesenteric artery, evidently draining the descending colon.

The drainage from the ovaries and testes is by vessels that accompany the correspondingly named arteries, first through one or two secondary nodes, then to the lumbar region, where these lymphatic trunks turn medially and run toward their terminal glands, which are situated ventral or ventro-lateral to the aorta and vena cava, extending from the bifurcation of the aorta to about the level of the posterior pole of the kidneys; that is, to pre-aortic, prevenous, and juxta-aortic glands in the lumbar region. To these same glands drains part of the lymph from the body of the uterus and Fallopian tubes in the female and from the epididymis in the male. These glands have been formed from the root of the genital branch as it comes off from the posterior end of the retroperitoneal sac (fig. 1, V. l. gon.). When the horns of the uterus are injected, the flow is both toward the cervix and peripherally toward the tubes. The medial portion of the horn and part of the cervix drain to a very large gland at the bifurcation of the external and internal iliac arteries. Thus the lymph from the sexual glands has only one channel of flow, i. e., to the glands that were formed from the posterior portion of the ventral or retroperitoneal sac. The lymph from the excretory canals of the sex-glands, on the other hand, has a double course of flow; that from the structures originating proximal to the sex-glands goes to the glands formed from the ventral sac, while the portions of the excretory canals distal to the sex-glands drain to glands arising from the primary lymphatics dorso-lateral to the aorta.

Injections of the bladder showed lymphatics draining to the external iliac glands. Drainage from certain regions of the bladder passed through intermediate lateral vesicle glands peripheral to the iliac nodes. Occasionally a vessel could be injected to the glands at the bifurcation of the aorta.

The lymphatics from the posterior trunk of the iliac sac become transformed into a chain of lymph-glands which are disposed fairly regularly about the blood-vessels (fig. 5, Lymgl. il. com.). In general, they are similar to those described by Poirier and Cuneo (1904), except that there is a characteristic ilio-inguinal node in the pig which is not seen in man.

The inguinal glands drain the superficial lymphatics over the lower abdomen, external genitalia, ventral side of the tail, the leg, and the medial side of the thigh. The efferents of the inguinal glands are distal external iliac nodes.

An ilio-inguinal node (fig. 5, Lymgl. ilio-ing.), characteristic of the pig, lies in the course of the ilio-lumbar artery near the crest of the ilium. It drains the superficial lymphatics of the posterior body-wall, down over the hip and the root of the tail (Sabin, 1904).

SUMMARY.

In the posterior half of the body, the first stage in the formation of a connected, well-developed primary lymphatic system in the pig is the presence of primary lymph-sacs, lying ventral and dorsal to the aorta. The ventral sac is the large retroperitoneal sac, which extends from the region of the cœliac axis to the bifurcation of the aorta. Dorsal, or dorso-lateral, to the aorta is the remainder of the primary system—the paired iliac sacs—which extend from the region of the adrenals to the bifurcation of the aorta.

According to their origin from these primary sacs, lymphatic vessels of the organs and structures in the abdominal and pelvic regions fall into two great groups, dependent upon whether they arise from the primary lymphatics ventral to the aorta, or from the primary lymphatics dorso-lateral to the aorta. As a general statement, it may be said that the ventral sac supplies the abdominal viscera, while the dorso-lateral sacs supply the retroperitoneal structures—the viscera, the body-wall, and the lower extremities—although, on account of considerable overlapping, this generalization must be regarded as only approximate.

Thus, from the retroperitoneal sac are given off lymphatics which drain the whole or part of the lung, diaphragm, liver and biliary passages, stomach, small and large intestine, capsule of spleen, pancreas, kidneys, Wolffian bodies, gonads, Müllerian and Wolffian ducts, and umbilical cord. The dorso-lateral lymphatics—that is, the paired iliac sacs—drain wholly or in part the diaphragm, body-wall, adrenals, kidneys, bladder, Müllerian and Wolffian ducts, umbilical cord, and the entire posterior half of the body. Only a few structures, notably the diaphragm, the kidneys, and the Müllerian and Wolffian ducts, which later become the excretory canals of the sex-glands, have lymphatics arising from both the ventral and the dorso-lateral sacs.

On following the development of the lymphatics in these posterior regions of the body, this early system of drainage is found to continue into the adult stage of lymphatic growth. The primary sacs change into lymph-glands which receive afferent vessels from the same organs and structures as did their parent sac. To a considerable extent the position of the primary lymph-glands, differentiated from the primary sacs, falls into two groups—the glands ventral and ventro-lateral to the aorta, and those dorsal and dorso-lateral to the aorta. Here, too, in the adult stage of lymphatic growth, the general statement can be made that the organs and structures situated within the abdominal cavity have their lymphatic drainage into the group of glands whose position is ventral or ventro-lateral to the aorta. On the other hand, those structures and organs lying outside of the abdominal cavity, as well as the entire posterior half of the body, have their lymphatic drainage eventually into the group of glands situated dorsal or dorso-lateral to the aorta.

ABBREVIATIONS.

Anas. ant.,	anterior anastomosis.
Anas. maj.,	major anastomosis.
Ao. abd.,	abdominal aorta.
A. il. com.,	common iliac artery.
A. umb.,	umbilical artery.
Cist. chy.,	cisterna chyli.
Diaph.,	diaphragm.
D. meson.,	mesonephric duct.
D. omph. mes.,	omphalo-mesenteric duct.
D. thor.,	thoracic duct.
Gl. suprar.,	suprarenal gland.
Gon.,	gonad.
Lig. caud. gon.,	caudal gonadal ligament.
Lig. cran. gon.,	cranial gonadal ligament.
Lig. diaph. meson.,	diaphragmatic ligament of the mesonephros.
Lig. genitoing.	genito-inguinal ligament.
Lymgl. coel.,	cœliac lymph-gland.
Lymgl. c. v. min.,	lymph-gland of lesser curvature of stomach.
Lymgl. d. choled.,	lymph-gland of biliary duct.
Lymgl. fundi,	lymph-gland of fundus.
Lymgl. gastroepip.,	gastro-epiploic lymph-gland.
Lymgl. gl. suprar.,	suprarenal lymph-gland.
Lymgl. hep.,	hepatic lymph-gland.
Lymgl. il. com.,	common iliac lymph-gland.
Lymgl. ilioing.,	ilio-inguinal lymph-gland.
Lymgl. juxta intest.,	juxta-intestinal lymph-gland.
Lymgl. juxta aorticæ,	juxta-aortic lymph-gland.
Lymgl. lien.,	splenic lymph-gland.
Lymgl. mes. sup.,	superior mesenteric lymph-gland.
Lymgl. praeaorticæ,	pre-aortic lymph-glands.
Lymgl. praeven.,	prevenal lymph-glands.
Lymgl. praevert.,	prevertebral lymph-glands.
Lymgl. retroven.,	retrovenal lymph-glands.
Meson.,	mesonephros.
Nod. ilioing.,	ilio-inguinal node.
Oe.,	œsophagus.
Or. uret.,	orifice of ureter.
Ovid.,	oviduct.
Pyl.,	pylorus.
Ramus retroren.,	retrorenal branch.
Rect.,	rectum.
Ren.,	kidney.
S. il.,	iliac sac.
S. il. pars caud.,	caudal part of iliac sac.
Sin. urog.,	urogenital sinus.
T. coel.,	cœliac trunk.
T. c. v. min.,	trunk to the lesser curvature of stomach.
T. gast. dex.,	right gastric trunk.
T. gastrolien.,	gastro-splenic trunk.
T. hypo.,	hypogastric trunk.
T. il. com.,	common iliac trunk.
T. il. ext.,	external iliac trunk.
T. mes. sup.,	superior mesenteric trunk.
T. post.,	posterior trunk.
Ur.,	ureter.
V. l. asc. duo.,	lymph-vessel of the ascending duodenum.
V. l. cap. gl. suprar.,	lymph-vessel of the suprarenal capsule.
V. l. caput pan.,	lymph-vessel of head of pancreas.
V. l. corp. pan.,	lymph-vessel of body of pancreas.
V. l. coli des.,	lymph-vessel of descending colon.
V. l. c. maj.,	lymph-vessel to greater curvature of stomach.
V. l. c. min.,	lymph-vessel to lesser curvature of stomach.
V. l. diaph.,	lymph-vessel of diaphragm.
V. l. fem.,	femoral lymph-vessel.
V. l. fundi,	lymph-vessel of fundus.
V. l. gl. suprar.,	lymph-vessel of suprarenal gland.
V. l. gon.,	gonadal lymph-vessel.
V. l. hep.,	hepatic lymph-vessel.
V. l. h. meson.,	lymph-vessel at hilum of mesonephros.
V. l. ing.,	inguinal lymph-vessel.
V. l. mes.,	mesenteric lymph-vessel.
V. l. pulm.,	pulmonary lymph-vessel.
V. l. pyl.,	pyloric lymph-vessel.
V. l. rect.,	lymph-vessel of rectum.
V. l. ren.,	renal lymph-vessel.
V. l. sacr. lat.,	lateral sacral lymph-vessel.
Vena omph. mes.,	omphalo-mesenteric vein.
Vena umb.,	umbilical vein.
Vent.,	stomach.
Ves. urin.,	urinary bladder.

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CONTRIBUTIONS TO EMBRYOLOGY, No. 59.

RELATIVE WEIGHT AND VOLUME OF THE COMPONENT PARTS OF THE BRAIN OF THE HUMAN EMBRYO AT DIFFERENT STAGES OF DEVELOPMENT.

By George B. Jenkins,
Department of Embryology, Carnegie Institution of Washington.

With twelve text-figures and one chart.

RELATIVE WEIGHT AND VOLUME OF THE COMPONENT PARTS OF THE BRAIN OF THE HUMAN EMBRYO AT DIFFERENT STAGES OF DEVELOPMENT.

INTRODUCTION.

The following volumetric study of the developing encephalon in the human embryo was undertaken as a supplementary part of a program of investigations in the morphology and differentiation of the central nervous system with which I have been engaged for several years. The work was made possible through the kindness of Dr. Streeter who, in furtherance of its purpose, has placed at my disposal the collection of embryos of the Carnegie Laboratory of Embryology, together with models, notes, and such other related material as has been accumulated. Under these conditions it was possible to secure a sufficient number of developmental stages for the investigation, including the consideration of the relative rate of growth of the various parts of the encephalon during the whole period of intrauterine life. It is with pleasure that I take this opportunity to acknowledge my indebtedness for the hospitality, aid, and interest that have been extended to me on the part of the staff of this laboratory.

The question of growth of the animal body as a whole and of its various component parts has occupied the minds of many investigators over a long period of time, and yet in the mass of literature which has resulted from these studies comparatively little is to be found that deals with the volumetric development of the central nervous system in the human embryo. A study of the literature on the subject of weight, size, degree of development, etc., of the brain reveals the fact that practically all of the observations reported have dealt with postnatal material and the great majority with adult brains, secured in the main from various institutions, such as asylums for the insane, almshouses, and homes for the aged. The great questions seem to have been those concerning body size, the attempted correlations between brain size and intellectuality, and the controversy over the differences in brain size in the two sexes. Not a few reports frankly deal with grossly pathological material. Much additional work, of anthropological value mainly, has been done along the lines of craniometry, notably by West (1894), who made a number of observations upon school children of both sexes, recording the head measurements as a part of the general growth conditions, Lewandowsky (1910), and Hrdlička (1919), who treat of the more pertinent question of the correlation between skull size and cranial capacity.

Aside from these latter cases the bulk of the work reported may be roughly grouped into four divisions:

(*a*) The study of brains of individuals of superior intellectual attainments (Spitzka, 1907; Marshall, 1892; Manouvrier, 1885).

(*b*) The tabulation of the brain-weights and measurements obtained from large series of cases, including material of various races and ages and representing both sexes (Boyd, 1861; Donaldson, 1895; Ziehen, 1899, 1903; Vierordt, 1893).

(*c*) Statistical studies in which these tables are used to determine the correlations existing between brain-size and age, brain-size and intellect, and the comparison of brain, body, and intellect in the two sexes at various ages and in different races, as has been so ably done by Pearl (1905*a*, 1905*b*) who, in addition to the above material, used the tables reported by Bischoff (1880), G. Retzius (1900), Marchand (1902), Matiegka (1903), and Boyd-Marshall, all as reported by Donaldson (1895). The employment of such biometric methods gives results of great value to the investigator, since it enables him to make a great number of comparisons and to tabulate and express his results with a clarity and an exactness not possible by any other method.

(*d*) This last class would embrace the relatively limited number of observations dealing with material sufficiently young to come within the scope of the present paper, and includes extracts from Major Boyd's (1861) monumental work on approximately 2,600 cases autopsied at Marleybone Workhouse and the Somerset Lunatic Asylum. These cases were grouped, according to age, into 18 periods, the first 3 of which are of value in this connection. They comprise 48 premature still-born, 83 term still-born, and 90 new-born infants. The various measurements as given by Boyd were the total body-length from the vertex to the inner side of foot behind the ball of the great toe, and the following head measurements: circumference around the occipital protuberance to the space behind the eyebrows; transverse, from the opening of one ear, over the vertex to the opening of the other. Doubtless the method of subdividing the encephalon employed by Boyd was, as assumed by Donaldson, for the purpose of making the cerebrum one unit, the cerebellum one unit, and the medulla, pons, and midbrain one unit; though it is probable that a part, at least, of the mesencephalon was included with the cerebrum, since, upon removing the calvarium and dura, Boyd "removed the right and left hemispheres of the cerebrum in slices to the tentorium." The cerebellum was isolated by cutting the crura close to the pons.

A closer study of the group of specimens tabulated as premature still-born, which are recorded as measuring 10 to 18 inches total length (CH), or approximately 250 to 460 mm., shows that they would correspond to an age range from the end of the fifth to the end of the eighth month, according to Stratz's tables as reported by Martin (1914). According to Mall (1910), these two extremes would for the younger correspond to a sitting-height (CR) of 167 mm., age 20 weeks; and for the older a sitting-height of 310 mm., age 35 weeks. The weights of the brain parts in these two extremes would be in the smaller fetus 41.25 grams for the cerebrum, 1.98 grams for the cerebellum, and 1.32 grams for the medulla-pons; and in the larger fetus 297 grams for the cerebrum, 24.75 grams for the cerebellum, and 1.65 for the medulla-pons. These figures must at best be accepted with reservations, since the tables from which they are taken are undoubtedly inaccurate in some particulars. For example, the weight of the encephalon in no case equals the added weights of

the various parts of the same brain. These results are probably due to the use of crude apparatus and to a tendency to give results in round numbers.

Ziehen (1903) includes 9 embryos in a large series of cases reported. The age of 6 of these was 30 days and the recorded total brain-weights were respectively 0.98, 1.0, 1.05, 1.13, 1.15, and 1.15 grams; 3 were 29 days old and the total brain-weights were 1.29, 1.23, and 1.15 grams respectively. All of the material has been preserved in formalin.

Michaelis (1907), in a number of cases of children autopsied by him in Leipzig, reports 7 groups of embryos. Jackson (1909), in a study of prenatal growth, records observations made upon 43 specimens ranging from 6 mm. to full term. In these studies the measurements were by volume and not by weight, as in the other cases recorded. The volume was determined in some cases by the amount of water displacement of the brain of the embryo modeled in wax by the Born method; in others by making enlarged drawings of the parts, measuring these drawings with a planimeter and calculating the volume by multiplying the areas by the thickness of the sections. His conclusions are that the brain, although subject to considerable variation, shows a fairly regular curve of growth. At the second month it forms slightly more than 20 per cent of the total body-volume, the average dropping to 12.8 per cent in still-born fetuses. In the living-born, however, the average was about 14.6 per cent.

Dockeray (1915), working toward the same end which is the purpose of my own paper, and using similar methods, gives a complete volumetric study of the brain parts in a fetus 156 mm. in length, and I have incorporated his results in this paper.

METHODS AND MATERIAL.

Two points in the technic employed in my own studies were determined upon because their worth had been proved, both in this laboratory and by other workers:

First, the use of the crown-rump or sitting-height as a standard for determining the size of the material used: Both Le Bon (1879) and Donaldson (1909) agree that body-length is a better criterion than body-weight from which to infer brain-weight. The use of this method relieves us of the necessity of determining the leg-length, an unsatisfactory procedure even in older fetuses.

Second, the method of preservation: All of the material used in this study had been preserved in formalin which alone in varying strengths, or in combination with other agents, is by far the most universally used preservative for animal tissues. Hrdlička (1906) experimented with the effects of various preservatives upon the brains of both human and lower vertebrates and found formalin the most satisfactory. Though confirming in part the work of others who claim that this agent causes an increase in volume in the tissues preserved in it, in his summary he states that the simple formalin solutions all show the same effects in all brains. These consist of a sharp initial rise in the weight of the specimen, reaching a maximum within less than a week, and a subsequent gradual, long-continued decrease. The rise is in inverse ratio to the strength of the formalin solution, the percentage of loss being apparently independent of the formalin percentage.

Many workers have tested out various fixatives with more or less indifferent success, and while for special tissues and under favorable conditions many satisfactory methods have been developed, still for general purposes, where availability of the agent and the ease with which it can be prepared are the main desiderata, none have yet been discovered which are as satisfactory and consequently as widely used as is formalin in varying degrees of strength.

In order to secure the necessary data upon which to base conclusions, a great number of embryos were studied and such stages selected as it was thought would best show the developmental phases under consideration. Out of this number, 10 specimens were chosen, ranging in size from 4.3 mm. CR, estimated age 4½ weeks, to a new-born infant of 367 mm. CR. All the material used is to be found in the Carnegie Embryological Collection. In table 1 will be found listed the embryos that were selected as being particularly suitable for the purposes of this study. They are arranged serially in the apparent order of their development. The catalogue numbers given are those under which the embryos are listed in the records of the laboratory. The measurements all signify the crown-rump length in millimeters. The ages in weeks were determined from the last known menstrual period and conform to the curve of age (based on length and weight) plotted by Streeter (1920). All of the specimens had been preserved in formalin, and the first 8, in addition to fixation, had been embedded, cut, and stained. The material was used as found, no attempt being made to account for any tissue changes dependent upon the technic. The volume is therefore that of the embedded specimen. The shrinkage resulting from the embedding process introduces a considerable error in connection with actual volume, but as the shrinkage of the component parts of a given brain is uniform, the error does not prevail in reference to the relative volume of the separate parts.

In each of these younger embryos the brain was projected, drawn, and modeled, according to the well-known method of Born. (See table 1 for magnifications used in each instance.) The resulting models could then be easily subdivided into the various parts decided upon and the weights and volumes of these readily obtained. The two older specimens were of a sufficiently advanced stage of development to permit of dissection and weighing of the actual tissue.

TABLE 1.—*List of specimens the brains of which were studied.*

Catalogue No.	Crown-rump length.	Estimated menstrual age.	Magnification of model.
	mm.	*weeks.*	*diameters.*
148	4.3	4½	50
43	16	7	20
584*a*	25	8	20
96	50	11	10
1400–20	69.5	12½	10
1400–22	80	13½	10
1400–25	119	16	5
1400–28	156	19	5
.....	230	26	Dissected
2558	367	40	"

The first question that arose was the selection of a method of subdivision which could be adapted to all stages and which would yet permit the greatest number of dissections and comparisons. Only simple determinations were possible in the early stages, additions being made as the encephalon increased in complexity. In seeking to establish definite landmarks by which to be guided in delimiting the various parts, it was decided to base the initial procedure upon the early subdivision of the encephalon into the primary brain vesicles and then to add such later subdivisions as could be accurately determined by a study of the gross models and a microscopic study of the tissues. This plan permitted the development of a logical method of procedure.

Since in all stages it was possible to delimit the three early vesicles—the rhombencephalon, mesencephalon, and prosencephalon—the first point to determine was that of landmarks by which these vesicles could be separated from each other and the hindbrain from the spinal medulla. In the latter, of the three criteria most commonly used—the highest root fibers of the first spinal nerve, the lowest root fibers of the hypoglossal nerve, and the lowest crossing fibers of the pyramidal decussation—the first proved to be the most satisfactory. Accordingly, the first section was made in a transverse plane immediately anterior or cranial to the highest rootlets of the suboccipital nerve. This section was the same in all cases, and the resulting anterior mass, the encephalon, was the portion used for these studies. The second section—the separation of the hindbrain from the midbrain—was made in the youngest specimen (4.3 mm. CR length) in the constriction immediately in front of the outward curve of the rhombic lip, the cerebellar rudiment. Later, when it was possible to discern the inferior colliculi and the superficial transverse pontine fibers, the incision was started dorsally, just posterior to the colliculi, to end ventrally immediately anterior to these pontine fibers.

The mesencephalon is much more prominent proportionally in the younger subjects than in the older, and forms the arch of the cephalic flexure. It is wedge-shaped, the ventral surface being concave and shorter, the dorsal surface convex and longer, and delimited in front and behind by transverse surface grooves. The posterior incision was comparatively easy and was made, as described above, to remove the rhombencephalon. The line of demarcation between the mesencephalon and the prosencephalon was, however, more difficult to follow, since it was found upon studying the tissues that the thickened lateral plates of the mesencephalon project forward into the prosencephalon or, more properly speaking, are invaginated as a result of the caudal growth of the prosencephalon, so that the line of incision must extend anteromesially between these mesencephalic plates and the more prominent overlapping masses of the thalamencephalon. This lateral thickening of the mesencephalic plates, which is more pronounced in the basal parts ventral to the iter (the site of the future tegmental nucleus), is sufficiently well marked in these early stages to prevent error. Later, other landmarks make their appearance; dorsally, the epiphysis, the habenular nuclei, the posterior commissure, and the increasing prominence of the posterior extremities of the thalami; anteriorly, the pulvinars; posteriorly, the differentiation of the superior colliculi;

and ventrally, the developing mammillary bodies. In older subjects, therefore, this anteromesial incision must, on each side, extend between the mesencephalon and the thalami, to end just caudal to the epiphyseal attachment dorsally and the mammillary bodies ventrally.

Having divided the entire encephalon into the three primary vesicles, the succeeding steps consist in separating these structures into their more prominent constituent parts. In the 4 mm. embryo it was not possible to accurately remove the cerebellar rudiment. In the 16 mm. embryo, in the case of the hindbrain, the cerebellum could be removed from the stem. In the early stages this was accomplished by removing the prominent anterior rhombic lip on either side, which corresponds to the cerebellar rudiment, as shown in figure 2. This mass, at first a prominent roll or lip, becomes more and more sharply defined in the succeeding stages and is delimited from the mesencephalon by the transverse groove mentioned above. Owing to the difficulty of accurately separating the pons and the medulla, they were considered as a unit mass. The mesencephalon was considered as a single mass throughout the series. The fore-brain was then subdivided into the telencephalon and the diencephalon. In the early stage this division was accomplished by cutting along the thalamic margin, which extends as a slight groove along the dorsal border of the optic evagination and its later developing stalk connected with the diencephalon. In the older specimens the separation was effected by cutting through the internal capsule between the thalamus and the corpus striatum dorsally, the stria terminalis being used as a guide; ventrally the incision was made along the lateral margin of the optic tract, leaving this structure connected with the thalamencephalon. Thus the diencephalon comprises the optic tracts, the thalamus, the epithalamus, and the hypothalamus (except the hypophysis, which could not be considered in all cases). The diencephalon was not subdivided. The telencephalon in all stages was subdivided into the neopallium and the archipallium. In the early stages the line of incision was made to include the shallow depression in the lumen of the ventral wall of the first vesicle, lateral to the lamina terminalis and anterior to the optic stalk. In the older specimens (16 mm. and upwards) this rudiment is larger and consequently more easily separated from the remainder of the vesicle. Here there is a considerable ventral prominence appearing lateral to the lamina terminalis anterior to the torus opticus. This comprises the olfactory bulb and tract. The latter extends along the lower or ventral margin of the depressed cortical area over the base of the corpus striatum. In older subjects (50 mm. and upwards) the anterior commissure, paraterminal body, fornix, and hippocampus could easily be made out by microscopic study and as they became differentiated they were removed and studied.

The method employed in isolating these various structures in the older modeled embryos was essentially the same as that followed in dissecting the brain of the term fetus, which was as follows: The cord was separated from the medulla at the conventional level (in these specimens the bodies had been injected with a 10 per cent solution of formalin and the brain, after removal, had been kept in the same solution until studied) and the upper part of each cerebral hemisphere removed

by a transverse incision made just above the level of the fibers of the corpus callosum. The occipital lobe on each side was then removed flush with the caudal margin of the splenium and the basal surfaces of the temporal lobes were pared away until the junction of the mesencephalon and diencephalon could be seen. The mesencephalon was then separated from the rhombencephalon by an incision passing just cranial to the transverse pontine fibers ventrally, and just caudal to the posterior colliculi dorsally. The mesencephalon was next removed by an incision passing anteromedially between the anterior colliculi and the pulvinars, just caudal to the epiphyseal stalk dorsally and the corpora mamillaria ventrally. Next, the olfactory bulbs and tracts were freed and removed by cutting each tract at its junction with the base of the hemisphere. The two hemispheres were then separated by cutting through the median sagittal plane of the corpus callosum, the lamina terminalis, chiasma, and the basal structures, the incision passing between the mammillary protuberances. The resulting blocks were treated alike; the corpus callosum was lifted up and carefully dissected off from the fornix, exposing that structure, as well as the thalamus, stria terminalis, and the corpus striatum. The remainder of the cortex was removed anteriorly, with the head of the caudate nucleus as a guide. Laterally, the cortex of the insula was pared down to the lenticular nucleus and the temporal lobe removed, leaving the hippocampus, the fimbriæ, and the fornix intact. Following the fornix around anteriorly, its anterior pillar and the mammillary and paraterminal bodies were removed together. The thalamencephalon was then separated from the corpus striatum by cutting through the internal capsule, following dorsally the line of the stria terminalis, and ventrally, cutting just lateral to the optic tract, leaving that structure connected with the diencephalon. The cerebellum was then separated from the remainder of the hindbrain by cutting through the peduncles flush with the hemispheres.

This method of subdivision gave 8 separate units for study: (1) the medulla-pons, (2) the cerebellum, (3) the mesencephalon, (4), the diencephalon, (5) the fornix and hippocampus, including the paraterminal and mammillary bodies, (6) the olfactory bulb and tract, (7) the telencephalon, and (8) the corpus striatum. In all of the specimens a regrouping of these units was made, giving us the following additional units: (1) the total hindbrain, which comprises both the cerebellum and the medulla-pons; (2) the total telencephalon which was subdivided into archipallium (including the olfactory apparatus) and the neopallium—that is, the remainder of the telencephalon, minus the corpus striatum, which in the more advanced specimens was considered as a separate unit. In each instance the total weight and volume of the encephalon were determined; then those of each of the subdivisions were ascertained and the percentages calculated from the totals. The actual weight and volume could both be determined in the case of the dissected specimens, while for the modeled specimens the weight and volume of the entire model, or of a part, were first determined. The weights of these wax models were simply carried through, no attempt being made to ascertain the actual weight, since the relative values would have been the same. The volume of the model was obtained by determining the volume of a gram of the wax used in making the model and multiplying

this by the weight of the part under consideration. The cubic centimeter value of the wax being thus obtained in grams, it was a simple matter to calculate the various amounts, percentages, etc. The actual volume was obtained by dividing the model volume by the cube of the magnification used in the reconstruction.

In making this study the companion parts of the two sides have in every instance been grouped together, thus giving one unit for comparison instead of studying each of these paired structures separately. In all cases, too, the percentage weight has been chosen as the unit of comparison, since this method gives a stable basis, whatever may have been the magnification used in the reconstruction, and would apply equally well when the actual weights were considered, as in the two dissected specimens. Weight, rather than volume, has been selected for comparison for the same reason that model weights were considered instead of attempting to calculate actual weights, since it was thought that by reducing the method employed to the simplest possible working basis the percentage of error would likewise be reduced to a minimum. It is to be remembered, also, that these percentage weights are of only relative value, for the various parts of the encephalon present a steady and consistent growth and development throughout the period of gestation, and that the decrease in percentage weight, recorded for certain parts, as opposed to increased percentages for others, means merely that those parts which present an increase in weight grow so much more rapidly that they outstrip their more sluggish neighbors in relative bulk, as will be seen in a study of both models and tissues.

In every case, except the two oldest specimens, a careful check was kept upon all steps by a microscopic study of the tissues. This was found to be especially valuable in determining the landmarks to guide the incisions. However, while occasional reference may be made to the degree of differentiation, it is the intention at this time to deal entirely with the question of growth in bulk or mass, without reference to histologic detail.

DESCRIPTIONS OF INDIVIDUAL SPECIMENS.

No. 148, 4.3 mm., estimated menstrual age 4½ weeks.

This embryo (see table 3 and fig. 1), which has been the subject of a number of studies by various investigators, presents some quite unusual features and was selected for the reason that, for a young stage, it presents the various developmental features to such a degree as to render it especially valuable as a basis upon which to build a serial study of the various parts of the encephalon. At this stage the central nervous system is distinctly tubular in character and the anterior neuropore is closed. Anterior and dorsal to the eye-stalks, and separated from them by a distinct fold, can be seen the olfactory region or archipallium, and more dorsally the neopallium, consisting at this stage of two faintly outlined evaginations, one to either side of the mid-line. The entire prosencephalon comprises 31.3 per cent of the weight of the encephalon and can be quite easily subdivided into the telencephalon, weighing 7 per cent, and the diencephalon, weighing 24.3 per cent. The further subdivision of the telencephalon yielded an archipallium of 4.2 per cent and a neopallium of 2.8 per cent. The cephalic flexure is well defined; its arch, rather narrow, is formed by the mesencephalon, which constitutes 14.3 per cent of the encephalon. The rhombencephalon at this stage is proportionately very large, comprising 54.4 per cent of the total weight of the encephalon.

A study of this vesicle shows that the lateral plates have a wide dorsal spread; the thin roof plate is closely applied to the overlying ectoderm and only a slight thickening is observed at the point of junction of the roof and lateral plate—the rhombic lip. The cerebellar rudiment is merely a thickened fold which stretches across the more anterior, cephalic portion of the dorsum or

roof, showing a tendency to overlap the constricted caudal extremity of the mesencephalon. The cerebellum is not sufficiently differentiated at this stage to permit of its delimitation. The site of the pontine flexure is not indicated at this stage, though both the cervical and the cephalic flexures are well marked.

No. 43, 16 mm., estimated menstrual age 7 weeks.

This specimen (see fig. 2) presents a much more advanced stage of development. The tubular character is less pronounced and the pontine flexure is well defined, serving to accentuate both the cervical and cephalic flexures. That portion of the rhombic lip constituting the cerebellar rudiment was sufficiently well differentiated to permit of its removal and study,

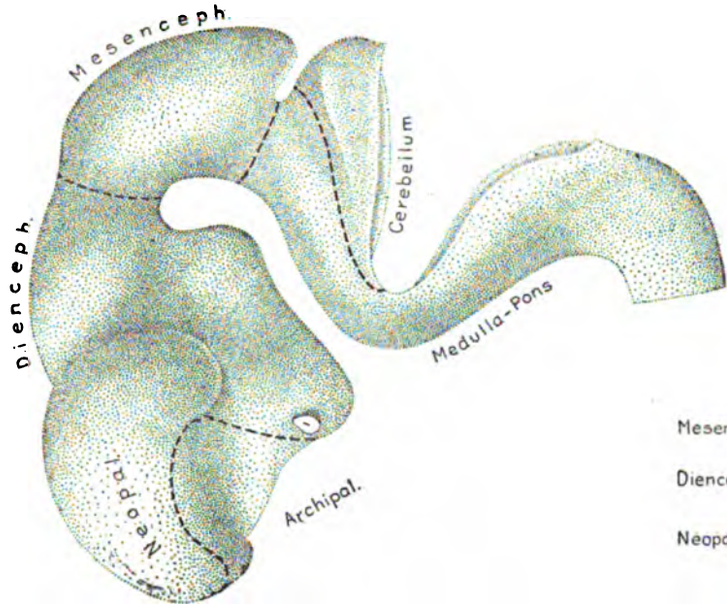


FIG. 2.—Left profile view of reconstruction of brain of embryo No. 43, crown-rump length 16 mm. × 10.

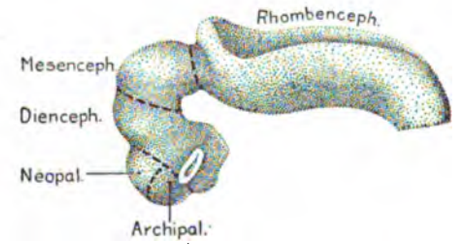


FIG. 1.—Left profile view of reconstruction of the encephalon of embryo No. 148, crown-rump length 4.3 mm., showing manner in which the brain was subdivided. × 25.

contributing 7.56 per cent of the total weight as opposed to 38.11 per cent for the medulla-pons, the combined weights giving a total of 45.67 per cent for the rhombencephalon, a relative decrease from the preceding specimen. The mesencephalon has decreased to 12.52 per cent and the diencephalon to 20.38 per cent, whereas the telencephalon has increased to 21.43 per cent, three times its weight in the younger embryo. Of this the archipallium constitutes 18.09 per cent and the neopallium 3.34 per cent, making a total of 41.81 per cent for the prosencephalon, as opposed to 31.3 per cent in the 4.3 mm. stage.

No. 584*a*, 25 mm., estimated menstrual age 8 weeks.

This embryo presents a still further increase in development, the rhombencephalon weighing 36.63 per cent of the total brain. Of this the cerebellum comprises 9.74 per cent and the medulla-pons 26.89 per cent, a slight increase for the latter. The mesencephalon has increased to 14.76 per cent, the total prosencephalon to 48 per cent, to which the diencephalon contributes 19 per cent—a slight shrinkage, and the telencephalon 28.84 per cent, a gain of 7 per cent over the preceding stage. A further study of the telencephalon shows an archipallium of 1.43 per cent, a tremendous drop as compared with 18.09 per cent at 16 mm. The neopallium has, on the other hand, increased to 27.41 per cent, a complete reversal of relative values.

The basal ganglia, both the thalami and the corpora striata, show a considerable increase in bulk at this stage. The walls of the vesicles also show a corresponding increase in thickness, so it will be observed that, although there has been a marked increase in size and weight of the encephalon up to this stage of development, it consists in cell proliferation rather than in dedifferentiation and does not permit of much added study of individual parts.

No. 96, 50 mm., estimated age 11 weeks.

This embryo (figs. 3, 4, and 5) has advanced much farther in development and permits of a greater amount of subdivision of the model. The total brain-weight is 514.58 grams, of which the rhombencephalon comprises 12.77 per cent, the cerebellum contributing 3.79 per cent and the medulla-pons 8.96 per cent, a relatively con-

siderable decrease in all three values. The mesencephalon has likewise decreased, weighing in this specimen but 7.09 per cent of the total, as compared with twice that amount in the 8 weeks' embryo. The total prosencephalon has nearly doubled its relative weight, being now 80.14 per cent, the diencephalon dropping to 12.77 per cent, and the telencephalon increasing to 67.37 per cent, of which the archipallium constitutes 3.52 per cent and the neopallium 51.18 per cent, well back over the diencephalon, encroaching upon the mesencephalon. A curious feature, noted only at this age, is that the corpora striata, the diencephalon, and the rhombencephalon, are all of equal weight value.

No. 1400–20, 69.5 mm., estimated age 12½ weeks

This specimen still more closely approaches the full adult differentiation. The cerebral hemispheres have grown in all directions to a con-

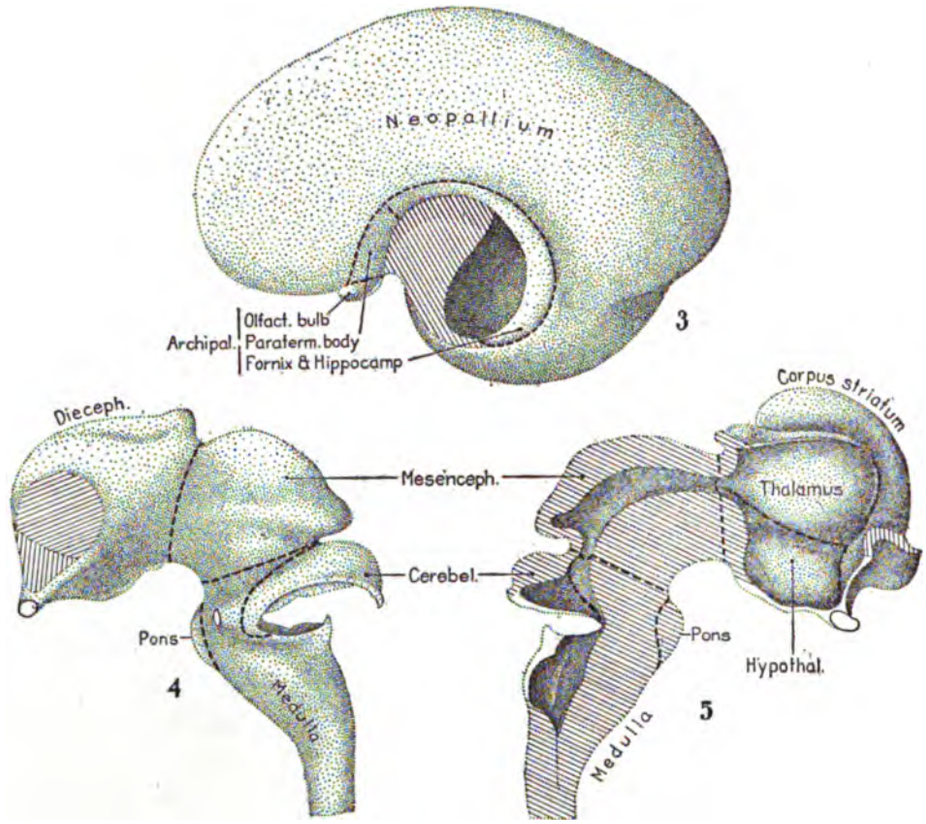


FIG. 3.—Mesial view of right hemisphere of embryo No. 96, crown-rump length 50 mm., showing subdivisions of archipallium. × 5.

FIG. 4.—Left view of model of brain-stem (same embryo as shown in fig. 3), showing its subdivisions. × 5.

FIG. 5.—Mesial view of hemisection of model shown in fig. 4, showing subdivisions of diencephalon. × 5.

a tremendous increase over all preceding stages. The corpora striata, which could be isolated in this specimen, weighed 12.67 per cent of the total brain. The various parts of the archipallium could also be isolated, giving an olfactory bulb weighing 0.3 per cent, and the paraterminal body, fornix, and hippocampus, which together weighed 3.22 per cent of the total. The pontine flexure is much less acute and the cerebellum, as yet rudimentary, is a narrow, beak-like structure (fig. 3). The mesencephalon shows a distinct caudal projection overhanging the median part of the roof of the cerebellar rudiment. The cerebral hemispheres project siderable degree, the total prosencephalon weighing 84.84 per cent of the whole. Of this the diencephalon contributes 11.47 per cent, a steady relative decrease, despite the increase in bulk of the developing thalami. The archipallium has relatively decreased, weighing but 2.87 per cent, of which the olfactory bulb contributes but 0.15 per cent and the paraterminal body, fornix, and hippocampus 2.72 per cent. The neopallium, on the contrary, weighs 60.62 per cent and the corpus striatum 8.88 per cent, making a total of 69 per cent of the entire encephalon. The mesencephalon has dropped to 5.97 per cent and the total rhombencephalon

to 10.19 per cent, of which the medulla-pons represents 6.23 per cent and the cerebellum 3.96 per cent, thus showing the continued and rapid increase of the prosencephalon, especially the neopallium, over the other parts.

No. 1400–22, 80 mm., estimated age 13½ weeks.

A still more decided advance in bulk and differentiation is here observed (fig. 6). The cerebral hemispheres are considerably more

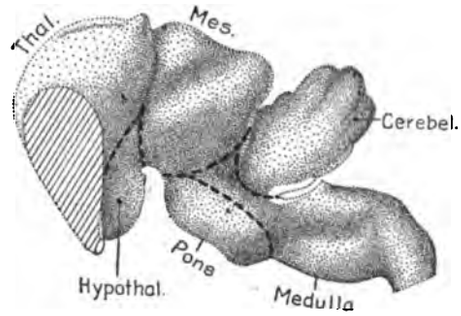
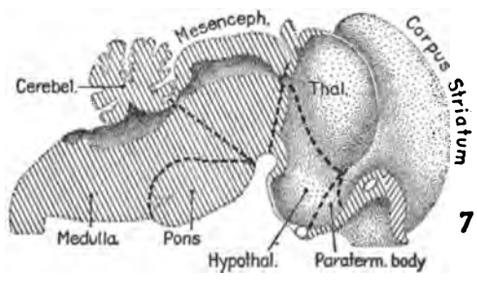


FIG. 6.—Left view of model of brain-stem, embryo No. 1400-22, crown-rump length 80 mm. × 6.

extensive, the telencephalon totaling 80.56 per cent of the entire weight of the encephalon. Of this the neopallium constitutes 65.55 per cent and the corpus striatum 11.23 per cent, a total of 76.78 per cent. The archipallium weighs 3.78 per cent, the olfactory bulb contributing 0.35 per cent and the paraterminal body, fornix, and hippocampus 3.43 per cent; all of which agree substantially with the figures given for the corresponding parts of the preceding embryo. On the other hand, the neopallium has increased and the diencephalon has diminished to 8.04 per cent, the total prosencephalon having increased to 88.6 per cent. The mesencephalon presents a sligh decrease, weighing now 4.78 per cent. The total rhombencephalon has also lost relatively, weighing 6.62 per cent, of which the cerebellum comprises 2.89 per cent, the medulla-pons 3.73 per cent.

No. 1400–25, 119 mm., estimated age 16 weeks.

The advance in general bulk is distributed in this embryo (figs. 7 and 8) as follows: The total prosencephalon constitutes 91.91 per cent of the brain weight, of which the diencephalon comprises 6.29 per cent—a decrease, the telencephalon having meanwhile increased to 85.62 per cent. To this the neopallium contributes 69.55 per cent, the corpus striatum 11.64 per cent, and the archipallium 4.43 per cent. Of the latter the olfactory bulb shows a decreased weight of 0.28 per cent, and the paraterminal body, fornix, and hippocampus a weight of 4.15 per cent—a slight rise. The mesencephalon has fallen to 2.06 per cent. The total rhombencephalon weighs 6.03 per cent, of which the cerebellum forms 2.74 per cent and the medulla-pons 3.29 per cent, all of which weights are practically identical with those of the corresponding parts in the 80 mm. specimen, so that the telencephalic gain is balanced by the losses sustained by the diencephalon and the mesencephalon, the fractional increase in the rhinencephalon being likewise accounted for.



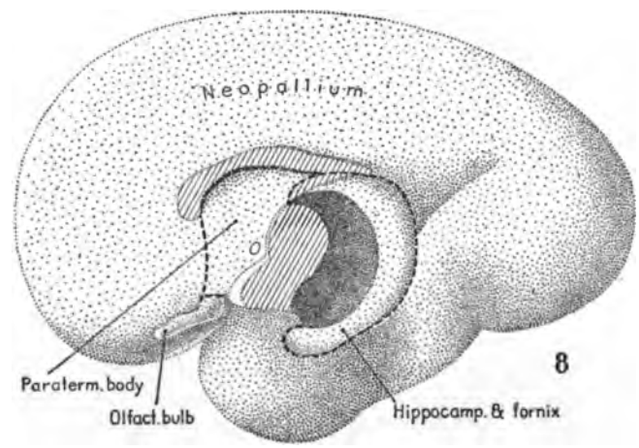


FIG. 7.—Mesial view of model of brain-stem of embryo No. 1400–25, crown-rump length 119 mm. × 2.5.
FIG. 8.—Mesial view of right half of telencephalon of embryo shown in figure 7. × 2.5.

No. 1400–28, 156 mm., estimated age 19 weeks.

This specimen (figs. 9, 10, 11, and 12) was studied and published by Dockeray (1915), and his findings have been rearranged and incorporated into this study. The total prosencephalon has attained the enormous weight-value of 93.69 per cent of the total brain weight, the diencephalon dropping to 4.81 per cent and the

telencephalon weighing 88.78 per cent. Of the latter the neopallium comprises 78.82 per cent and the corpus striatum 7.62 per cent, the former an increase, the latter a decrease as compared with the preceding stage. The archipallium has declined to 2.34 per cent, the olfactory bulb weighing 0.14 per cent, the paraterminal body, fornix, and hippocampus 2.2 per cent, the lowest point yet reached by these parts. The mesencephalon has also decreased, constituting but 1.44 per cent of the encephalon. The total rhombencephalon weighs 4.97 per cent, slightly below that of the 119 mm. specimen, the loss being sustained by the medulla-pons which now weighs 2.23 per cent, while the cerebellum weighs 2.74 per cent, the same weight-ratio as that in the preceding stage and virtually the same as that in the 80 mm. stage. This structure thus maintains a relative weight-level extending over a period of six weeks, which really means that there has been an acceleration of growth during this interval. The foregoing specimens were all modeled and the models separated into these various parts for study.

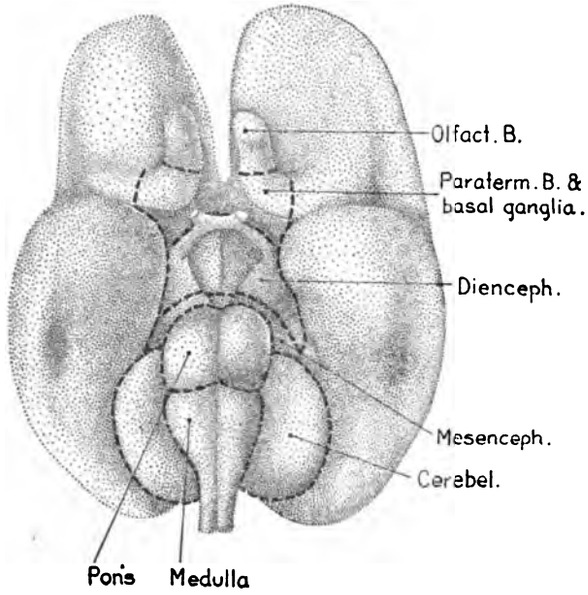


FIG. 9.—Ventral view of a reconstruction of brain of embryo No. 1400–28, 156 mm. crown-rump length, showing manner in which it was subdivided. × 2.

Fetus 230 mm., estimated age 26 weeks.

This stage is represented by a fetus of which only the brain was available for study. Since the specimen had reached a sufficiently advanced state of development to permit the removal and dissection of the brain, the actual weights and volumes can be given. In form and fissures this brain corresponds to a stage between the two shown by Retzius (1896, plate 14, figures 3, 4, 5, and figures 6, 7, 8), and has therefore been placed at 340 mm. total length, or 230 mm. crown-rump length, and its age estimated as 26 weeks. These data were obtained from Dr. Streeter, who dissected and studied the specimen.

The total prosencephalon weighed 93.32 per cent of the encephalon, a fractional loss as compared with the 156 mm. fetus. Of this the diencephalon constituted 3.7 per cent, less than the preceding one. The telencephalon, the total weight of which was 89.62 per cent, was subdivided into the neopallium, weighing 79.21 per cent, and the corpus striatum, weighing 8.33 per cent, both showing an increase. The archipallium weighed but 2.08 per cent, the lowest point yet reached. To this the olfactory bulb contributed 0.13 per cent, the paraterminal body, fornix, and hippocampus, 1.95 per cent. The mesencephalon weighed 1.42 per cent, practically the same as in the preceding specimen. The total rhombencephalon weighed 5.26 per cent, a somewhat greater relative weight than at 19 weeks. This added amount was contributed by the cerebellum, which weighed 3.15 per cent, as compared with a medulla-pons weight of 2.11 per cent, which is only a small fraction less than in Dockeray's fetus, the last and largest of the modeled specimens.

No. 2558, 367 mm., new-born white male.

This specimen had been injected and preserved in 10 per cent formalin. The sitting height was 367 mm., body-weight 3,210 grams. The head measurements were as follows: length 130 mm., width 102 mm., circumference 381 mm., biaural arc 248 mm. The brain was removed by Dr. A. H. Schultz, of the Carnegie Embryological Laboratory, to whom I am indebted for the foregoing data. Judging from macroscopic appearance, both body and brain were normal and well developed. The encephalon weighed 505.61 grams; the actual volume, as determined by water displacement in a large graduated cylinder where the indices could be easily read, was 489.97 c. c. The cerebral hemispheres were very large, richly convoluted, and had thickened until the cavity of the lateral ventricle was reduced to a mere slit. The total prosencephalon weighed 467.12 grams, or 92.38 per cent of the whole brain, slightly less than that of the preceding. Of this the diencephalon contributed 2.63 per cent, a loss of about 30 per cent as compared with the 230 mm. fetus. The telencephalon weighed 89.75 per cent of the whole, maintaining the same weight-value as the preceding specimen. Of this the neopallium weighed 424.3 grams, or 83.92 per cent, and the corpus striatum 24.3 grams, or 4.8 per cent, a considerable relative gain for the former. The archipallium

has declined to 5.2 grams or 1.03 per cent, which is the lowest mark in the weight-curve for this complex. Of this the olfactory bulb contributed 0.2 per cent, and the paraterminal body, fornix, and hippocampus, 0.83 per cent. The mesencephalon likewise reaches its lowest relative level at this stage, weighing but 0.64 per cent of the total weight of the encephalon. The rhombencephalon weighed 35.28 grams, or 6.98 per cent, the medulla-pons weighing 4.58 grams (0.91 per cent) and the cerebellum 30.7 grams (6.07 per cent). This represents for the former the lowest point in the series, and for the latter a marked gain over preceding figures. In this specimen the total brain constituted 15.75 per cent of the body-weight, a somewhat greater proportion than is usually reported. Jackson (1909) gives 14.6 per cent and Vierordt (1893) 12.29 per cent.

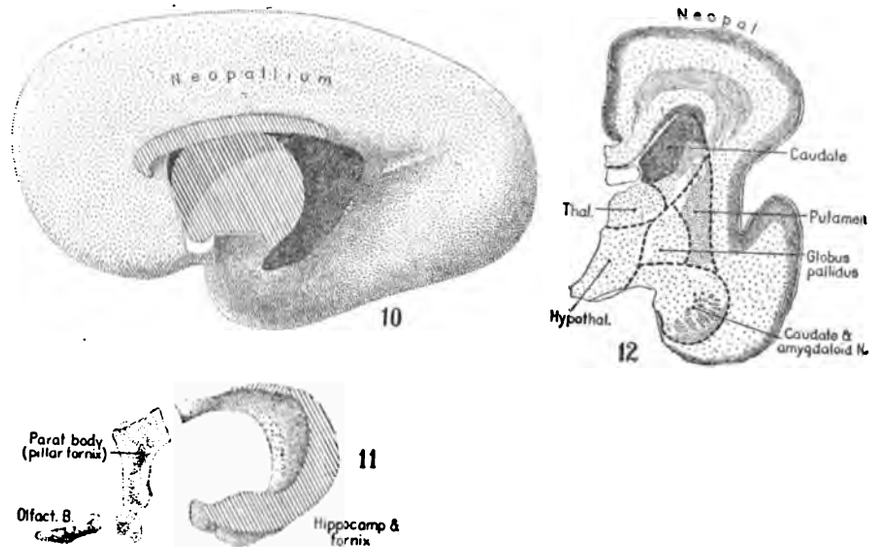


FIG. 10.—Mesial view of right half of telencephalon of embryo shown in fig. 9. × 2.
FIG. 11.—Mesial view of the subdivided archipallium of embryo shown in figs. 9 and 10. × 2.
FIG. 12.—Drawing of a hemisection of brain shown in figs. 9, 10, and 11, with subdivisions of basal ganglia outlined. (Section *b*, slide 145.) × 2.

BRAIN VOLUME AT DIFFERENT STAGES.

Since we have the volume of the models of the brain and its separate parts for the different stages of development, it is possible to roughly calculate the actual volume by dividing the model volume by the cube of the magnification used for the reconstruction. It must be borne in mind, however, that since we are dealing with brains that have been embedded in paraffine or celloidin, the models represent the embedded brain and one must take into account a considerable shrinkage of the tissues. The shrinkage of a given brain is more or less uniform throughout and therefore, in itself, does not interfere with the determination of the relative volume of its different parts. As regards the actual volume, however, one must take into consideration the primary swelling of the brain when placed in a formalin solution and its subsequent shrinkage in the course of its preparation into serial sections. From our own experience and that of others we can estimate

TABLE 2.—*Volume of brain at different stages of development, calculated by dividing the model volume by the cube of its magnification.*

Age.	CR length.	Volume of brain.
weeks.	*mm.*	*c. c.*
4	4	0.003
7	16	.041
8	25	.126
11	50	.603
12½	69.5	1.977
13½	80	1.503
16	119	8.750
19	156	13.120
26	230	54.290
40	367	489.970

that the volume of the brain as embedded is from one-tenth to one-third less than the original, depending on the character of the tissues and their reaction to formalin, and on the dehydrating and embedding media. The extent of this shrinkage for the individual brains I have at present no way of accurately determining. In spite of the fact that my figures are only approximate (probably one-

TABLE 3.—*Data on the relative weight and volume of the subdivisions of the brain at various stages of development.*

With the exception of the two oldest stages, these data are based on wax-plate reconstructions. Where the structures are bilaterally symmetrical the data for the right and left are combined.

	Rhombencephalon	Medulla-pons	Cerebellum	Mesencephalon	Diencephalon	Telencephalon	Neopallium	Corpus striatum	Neopallium and Corpus striatum.	Archipallium	Hippocampus, fornix and paraterminal body.	Olfactory bulb	Total brain
Embryo 4.3 mm. CR, No. 148. Brain modeled × 50 by Streeter.													
Model weight, grams	19.31	.....	.....	5.08	8.63	2.48	.99	.....	.....	1.49	.....	.....	35.50
Percentage of total weight	54.40	.....	.....	14.30	24.30	7.00	2.80	.....	.....	4.20	.....	.....	100.00
Model volume, c. c.	22.60	.....	.....	5.94	10.10	2.90	1.16	.....	.....	1.74	.....	.....	41.54
Embryo 16 mm. CR, No. 43. Brain modeled × 20 by Streeter.													
Model weight, grams	137.62	114.83	22.79	37.73	61.41	64.56	10.07	.....	.....	54.49	.....	.....	301.32
Percentage of total weight	45.67	38.11	7.56	12.52	20.38	21.43	3.34	.....	.....	18.09	.....	.....	100.00
Model volume, c. c.	150.00	125.16	24.84	41.12	66.94	70.37	10.97	.....	.....	59.40	.....	.....	328.43
Embryo 25 mm. CR, No. 584*a*. Brain modeled × 20 by Jenkins.													
Model weight, grams	325.10	238.70	86.40	131.00	175.50	256.00	243.30	.....	.....	12.70	.....	.....	887.60
Percentage of total weight	36.63	26.89	9.74	14.76	19.77	28.84	27.41	.....	.....	1.43	.....	.....	100.00
Model volume, c. c.	325.10	238.70	86.40	131.00	175.50	256.00	243.30	.....	.....	12.70	.....	.....	887.60
Embryo 50 mm. CR, No. 96. Brain modeled × 10 by Snyder & Sherrick.													
Model weight, grams	65.65	46.10	19.55	36.55	65.70	346.68	263.40	65.18	328.58	18.10	16.56	1.54	514.58
Percentage of total weight	12.77	8.96	3.79	7.09	12.77	67.37	51.18	12.67	63.85	3.52	3.22	.30	100.00
Model volume, c. c.	76.81	53.94	22.87	42.76	76.87	405.61	308.18	76.26	384.44	21.18	19.37	1.81	602.06
Fetus 69.5 mm. CR, No. 1400–20. Brain modeled × 10 by Jenkins.													
Model weight, grams	176.67	108.00	68.67	103.50	199.00	1255.24	1051.40	154.10	1205.50	49.74	47.10	2.64	1734.50
Percentage of total weight	10.19	6.23	3.96	5.97	11.47	72.37	60.62	8.88	69.50	2.87	2.72	.15	100.00
Model volume, c. c.	176.67	108.00	68.67	103.50	199.00	1255.24	1051.40	154.10	1205.50	49.74	47.10	2.64	1734.50
Fetus 80 mm. CR, No. 1400–22. Brain modeled × 10 by Sherrick.													
Model weight, grams	85.77	48.31	37.46	61.93	104.13	1043.70	849.23	145.46	994.69	49.01	44.40	4.61	1295.53
Percentage of total weight	6.62	3.73	2.89	4.78	8.04	80.56	65.55	11.23	76.78	3.78	3.43	.35	100.00
Model volume, c. c.	99.49	56.04	43.45	71.84	120.79	1210.69	985.11	168.73	1153.84	56.85	51.50	5.35	1502.81
Fetus 119 mm. CR, No. 1400–25. Brain modeled × 5 by Snyder.													
Model weight, grams	57.76	31.48	26.28	19.75	60.33	820.80	666.75	111.60	778.35	42.44	39.73	2.71	958.64
Percentage of total weight	6.03	3.29	2.74	2.06	6.29	85.62	69.55	11.64	81.19	4.43	4.15	.28	100.00
Model volume, c. c.	65.85	35.89	29.96	22.52	68.78	935.70	760.09	127.23	887.32	48.38	45.30	3.08	1092.85
Fetus 156 mm. CR, No. 1400–28. Brain modeled × 5 by Dockeray.													
Model weight, grams	72.14	32.33	39.81	20.90	69.76	1287.67	1143.20	110.61	1253.81	33.86	31.89	1.97	1450.47
Percentage of total weight	4.97	2.23	2.74	1.44	4.81	88.78	78.82	7.62	86.44	2.34	2.20	.14	100.00
Model volume, c. c.	80.57	36.50	44.07	23.66	78.97	1456.65	1294.10	124.21	1418.31	38.34	36.10	2.24	1639.85
Fetus 230 mm. CR, Brain dissected by Streeter.													
Actual weight, grams	2.91	1.17	1.74	.78	2.05	49.52	43.77	4.60	48.37	1.14	1.07	.07	55.26
Percentage of total weight	5.26	2.11	3.15	1.42	3.70	89.62	79.21	8.33	87.54	2.08	1.95	.13	100.00
Actual volume, c. c.	2.86	1.15	1.71	.77	2.01	48.65	43.00	4.52	47.52	1.13	1.06	.07	54.29
Fetus 367 mm. CR, No. 2558. Brain dissected by Jenkins.													
Actual weight, grams	35.28	4.58	30.70	3.21	13.32	453.80	424.30	24.30	448.60	5.20	4.20	1.(est)	505.61
Percentage of total weight	6.98	.91	6.07	.64	2.63	89.75	83.92	4.80	88.72	1.03	.83	.20	100.00
Actual volume, c. c.	34.18	4.43	29.75	3.11	12.91	439.77	411.18	23.55	434.73	5.04	4.07	.97	489.97

tenth to one-third less than the original volume), the interest attached to the question of the volume of the fetal brain perhaps warrants my including the data given in table 2, which were obtained on the basis of the volume of the models. From these same figures one can readily calculate the volume of any individual part of the brain by multiplying the total volume by the percentage of the total brain formed by that particular part (table 3).

SUMMARY.

A study of the preceding data shows the rapid growth of the brain as a whole and the relatively enormous rate of growth of some of its component parts as compared with others; for, while all parts of the brain consistently increase in size, they do not all grow at the same rate. The telencephalon, for example, shows a relatively rapid increase throughout the entire series, greatly surpassing all other parts; the cerebellum shows a similar, though less marked increase during the latter half of gestation; while all of the other parts show a relative decrease in weight-values.

Starting with an embryo of 4½ weeks, 4.3 mm. long, with an actual total brain volume of approximately 0.003 c. c., and in which it is possible to definitely outline little more than the three primary brain vesicles common to all vertebrates, one can follow the rapid growth and development until birth, when this complex and highly specialized organ attains a total actual volume of 490 c. c., a gain in less than 36 weeks of over 160,000 times the initial volume.

An analysis of the values for the different parts shows a steady upward curve for the prosencephalon, from 31.3 per cent in the early embryo to 92.38 per cent at term, a gain of nearly three times its initial bulk, despite the fact that all of its component parts, except the telencephalon, have shrunken considerably in weight-values. The percentage of the total brain-weight formed at different stages by the five chief subdivisions of the brain is shown graphically in chart 1.

The most striking feature in this growth curve, as well as the most significant one, is to be found in the enormous proportional and actual increase in the size of the telencephalon, which gives the amount of cortical expanse necessary to provide for the control of all subsidiary parts of the nervous system, as well as being the seat of the higher psychic functions. Beginning with an initial weight of 7 per cent at 4½ weeks, the telencephalon increases rapidly up to 13½ weeks, when it constitutes 80.56 per cent of the entire encephalon. Then follows a period of more gradual growth up to the twenty-sixth week, when the telencephalon attains its maximum relative weight (89.62 per cent) which it maintains until term. Assuming that the specific gravity of the brain tissue is very little over 1.0, the actual increase in weight is from approximately 0.0002 gram (as calculated from volume) at 4½ weeks to 493.8 grams at birth.

Another noteworthy point in the growth rate of the cerebral hemisphere is that it attains its maximum relative weight before any folding occurs to increase the extent of its periperhal surface, showing that in the later weeks its development is one of complexity rather than of bulk, a fact which would appear to argue that these later changes consist in perfecting the development of already existing structural units, rather than in the acquisition of new ones.

These figures for the telencephalon are rendered even more striking when we study the growth conditions of its various subdivisions. It was possible in all cases to separate this structure into the neopallium and the archipallium. At 4.3 mm. the undivided neopallium weighed 2.8 per cent; at 25 mm. 27.4 per cent; at 50 mm. 51.18 per cent, the curve continuing to mount rapidly to a maximum of 83.92 per cent at term. At the stage of 50 mm. it was possible to isolate the corpus striatum which weighed 12.67 per cent. From this point its weight-curve declines steadily to a minimum of 4.8 per cent at term. The archipallium at 4.3 mm. weighed 4.2 per cent, twice the initial weight-value of the neopallium, reaching a maximum of 18 per cent at 7 weeks, from which point the weight-curve declined steadily to 1.03 per

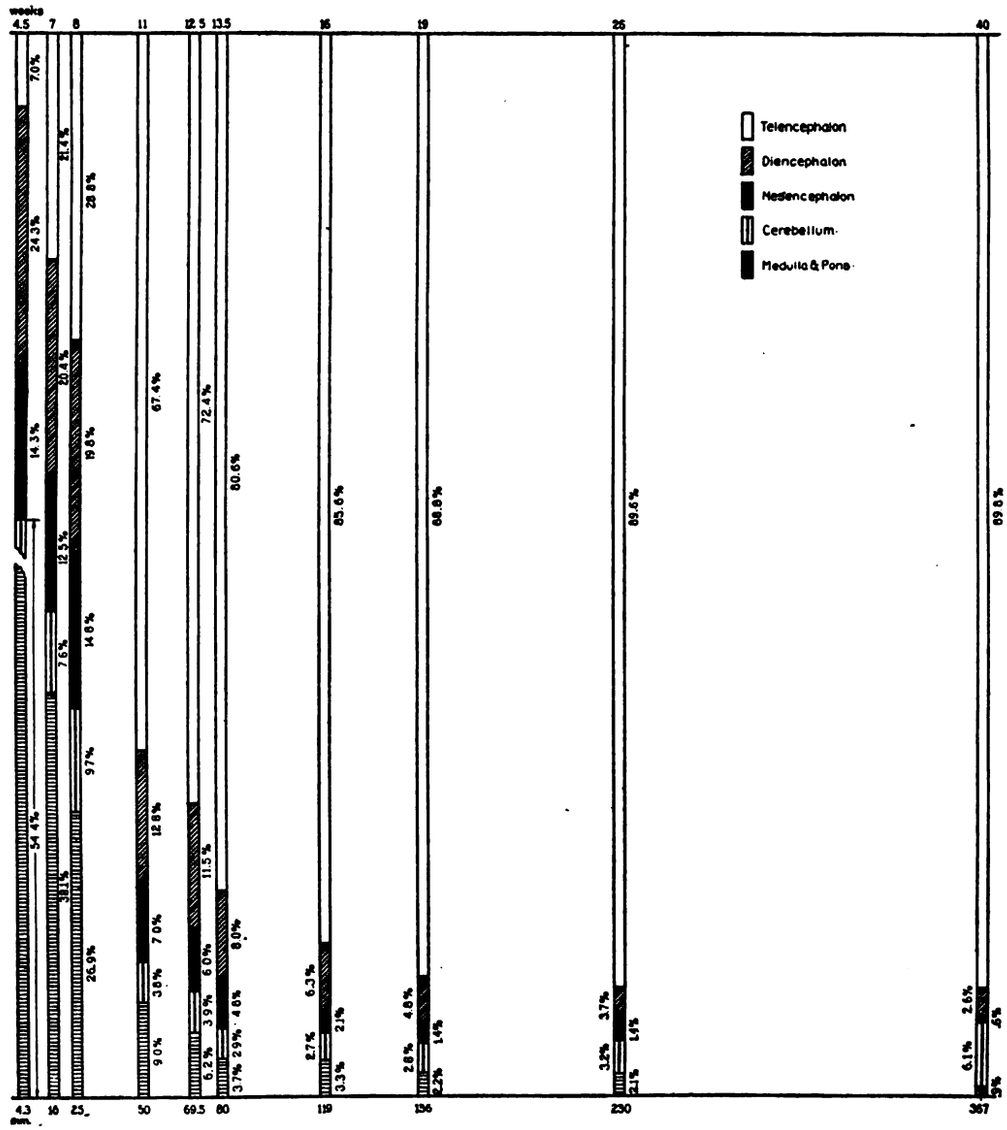


CHART 1.—Diagrammatic representation of the percentage weights of the brain parts at different stages of development.

cent in the new-born. At 11 weeks the archipallium could be subdivided into the olfactory bulb, which weighed 0.3 per cent, and the paraterminal body, fornix, and hippocampus, which together weighed 3.22 per cent. Both of these units show a consistent shrinkage in relative weight until birth, when the former weighed 0.2 per cent and the latter 0.83 per cent.

The diencephalon could also be isolated in all cases, and in the 4.3 mm. embryo had a weight-value of 24.3 per cent, or more than three times that of the telencephalon. From this high initial percentage the curve declined gradually to 2.63 per cent at term, notwithstanding the considerable increase in the size of the thalami.

The mesencephalon, weighing in the youngest specimen 14.3 per cent, drops steadily to 0.64 per cent at term, declining from a structure of considerable size to a mere slender, connecting stem, which attains its chief differentiation through the development of secondary centers connected with the fiber systems passing through it.

The total rhombencephalon starts with a maximum weight of 54.4 per cent of the whole in the 4.3 mm. specimen, from which point its curve sinks rapidly until at 156 mm. it reaches a minimum of 4.97 per cent, then rises gradually to 5.26 per cent at 230 mm., and 6.98 per cent at term. This unusual curve is explained when we study the growth-rate of the two component units of the rhombencephalon, which behave in a widely different manner. The curve for the cerebellum begins in the 16 mm. embryo at 7.56 per cent, ascends to 9.74 per cent at 8 weeks, its maximum point during prenatal growth. From this level it falls to 2.74 per cent at 13½ weeks, remaining there until the end of the nineteenth week when it rises rapidly, reaching 6 per cent at term, its actual weight being 30.7 grams. This reversal of the usually observed conditions shows the increasing importance of the cerebellum at these stages of development, its individual growth-rate becoming sufficiently marked to maintain an unvarying level during six weeks of changing values in other parts. It then gains a new impetus which is sustained throughout of the remainder gestation. The medulla-pons in the 16 mm. embryo comprises 38.11 per cent of the weight of the entire encephalon. The curve falls rapidly to 3.73 per cent at 13½ weeks, then more gradually to its minimum of 0.91 per cent at term, the general weight-curve for this part being comparable only to that of the diencephalon, though the relative loss is much greater in the medulla-pons. It would seem probable that the early large size of the medulla-pons is due to its association with the development of the cranial nerves which are relatively very large at this time.

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CONTRIBUTIONS TO EMBRYOLOGY, No. 60.

ABNORMALITIES OF THE MAMMALIAN EMBRYO OCCURRING BEFORE IMPLANTATION.

By George W. Corner,

From the Anatomical Laboratory, Johns Hopkins Medical School.

With two plates and one figure.

ABNORMALITIES OF THE MAMMALIAN EMBRYO OCCURRING BEFORE IMPLANTATION.

The occurrence of monstrosities, degenerative changes, and other abnormal conditions of the mammalian embryo brings up so many questions of scientific and practical interest, attended by such difficulties of approach, that it seems in order to report some recent observations which, though few in number, afford evidence of a positive character with regard to one of the points now in dispute.

Mall, in his several contributions on the nature and significance of abnormalities of the human embryo, never hesitated to assert the opinion that all these changes could be attributed to disturbances of nutrition due to faulty implantation of the ovum in the maternal uterine mucosa. This conviction was based on two grounds; first, that defective implantation could actually be observed in many of the abnormal human embryos which had come into his hands; second, that experimental teratologists had produced in amphibia, by the application of chemical poisons which presumably hindered the embryonic nutrition, all sorts of abnormalities resembling many of the known mammalian terata.

This hypothesis has never been subjected to critical test in the human species, because of the lack of specimens from stages prior to implantation; but the possibility of attacking it on logical grounds was seized by Kellicott, who pointed out (partly on the basis of his own remarkable experiments on the production of abnormalities in fish embryos by the action of low temperatures) that the onset of such changes might date from the earliest stages of cleavage of the ovum. He suggests that the cause of abnormal and monstrous development is to be found in disturbance of the normal organization of the ovum and he definitely considers the possibility that in mammals the disturbing cause might be found operative before as well as after implantation.

A year before Kellicott's contribution Huber had published an account of a few observations on abnormalities in very young embryos of the albino rat. His series included cases of separation of the first two blastomeres into half-embryos, incomplete or retarded segmentation, degeneration of ova at the end of the segmentation period, and abnormal formation of the segmentation cavity, all occurring in embryos which were lying free in healthy uteri. Since faulty implantation could be excluded, Huber was inclined to consider these abnormalities due to disturbances inherent in the ova.

The specimens described herein were obtained during the collection of a series of early embryo pigs. Figure 1 shows two of these embryos that were found among others in a healthy, normal uterus. One (A) is a normal blastodermic vesicle which would appear, by comparison with Assheton's well-known stages, to be about 7 days old; figure 7A, plate 2, illustrating the same ovum, displays its delicate, filmy texture, with clearly outlined nuclei, and the developing embryonic area below the

ectoblast. It is also shown in section in figure 2, plate 1. The other vesicle (B) is collapsed and wrinkled, its texture granular and almost opaque (the opacity is slightly exaggerated in fig. 7B), the nuclei hardly visible. Microscopic section confirms the external appearance of degeneration, showing the cells to be granular and their relations distorted (fig. 3). It is interesting to note that the preservation of the cells is better at one pole of the vesicle than at the other. In the ovaries of this sow there were 7 corpora lutea; in the uterus 6 vesicles, of which 2 were entirely normal, 2 normal in texture but collapsed and cup-shaped, and 2 abnormal, as illustrated. In addition there was 1 unsegmented ovum, thus accounting for all the ruptured follicles.

Figure 4 shows another case of the same sort with cupping of the vesicle and a partial break-down of the cells. This was obtained from another healthy uterus containing 1 normal vesicle and 4 of the collapsed type.

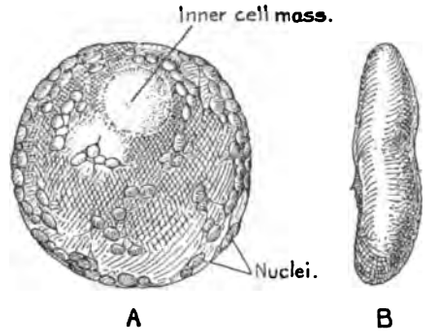

FIG. 1.—A normal (A) and a pathological (B) ovum obtained from the same uterus. The more detailed structure of these specimens is shown in figure 7, plate 2. The pathological ovum is wrinkled and compressed. Here it is shown from a side view; in figure 7 its broader surface is shown.

A third sow contained a still more interesting series of 4 vesicles. These were 5 mm. in diameter, a size attained by normal pig embryos at about the twelfth day, according to the studies of Assheton. In 3 the abnormality consisted of total absence of an embryonic area; in order to make sure of this point the vesicles were fixed in the fully dilated condition by injection of Bouin's fluid. I am indebted to my colleague, Dr. C. H. Heuser, for friendly aid in the preparation of these specimens. When dilated the 3 vesicles were of even thickness at all points of their walls. Microscopic section of one of them showed it to be composed of the usual two layers of cells.

The fourth specimen from this uterus showed, on the contrary, a distinct mass, slightly larger than the embryonic area found in a normal vesicle of the same dimensions, but opaque, white, and projecting from the surface of the vesicle by a pedicle, so that altogether it was not unlike a little mushroom in appearance. Figure 6 shows a section of this curious specimen, in comparison with a normal embryo from a vesicle of the same size (fig. 5). The little tumor is composed of cells of diverse staining reaction, disposed in an irregular way, but including three or four minute vesicular cavities, one of which appears in the section. The whole arrangement rather suggests a malignant papilloma; whether the tumor is at the site of the embryonic area itself or at some other part of the vesicle (the embryonic area being absent, as in the other blastodermic vesicles in the same uterus) can not

be decided; but in any event the specimen may be regarded as a true monster, the earliest ever seen in a mammal. This and the other related blastodermic vesicles, like those described in the previous paragraphs, were found lying unattached in the cavity of a healthy uterus which was quite normal and which proved on microscopic examination to be lined by a mucosa in every way similar to that of uteri containing normal embryos. The Fallopian tubes presented no gross abnormalities; they were not examined microscopically.

In view of the positive evidence afforded by the few specimens gathered by Huber and the writer, we are forced to the conclusion that the organism is liable to pathological changes before its attachment to the uterus. Whether these are due to faults inherent in the germ-cells, or to traumata which assail the ovum during its passage from the ovary to the uterine mucosa (such, for example, as chemically abnormal secretions of a seemingly normal uterus) our specimens of course do not explain; they merely increase the difficulty of the problem by demonstrating the inadequacy of the theory of faulty implantation to account for all developmental aberrations. Most likely it will be proved in the end that the germ-cells and their product are liable to the onset of abnormalities at all stages of their history; certainly hints of such a possibility are given by two lines of investigation, as yet but little applied to mammalian forms. The first of these comprises those experiments already mentioned, which show that the eggs of fish and frogs are susceptible to damage by all sorts of chemical and physical factors in their environment, at stages of development so early as to be comparable with the segmenting mammalian ovum during its passage through the tube and while in the uterus before implantation; the other line of approach is through the studies of Morgan and others, who have discovered the occurrence in plants, insects, and even mammals, of inheritable factors which, when brought into play by some unfavorable mating, cause such deviation from the anatomical or physiological norm as to kill the individual at one or another stage of its development. For instance, according to Cuénot, Castle, and Little, it is impossible to breed mice which are homozygous for yellow coat-color; it appears that the inheritance of this character through both parents is invariably fatal to the embryo; and, indeed, the recent report of Kirkham hints strongly that in this case the lethal factor may attack the embryo shortly before the time of implantation, while it is still in the morula or vesicle stage. It seems not impossible, therefore, that in future the application to mammals of both the teratological and the genetic modes of experimentation may aid in solving the complex but pressing questions arising from abnormalities of development of the human organism.

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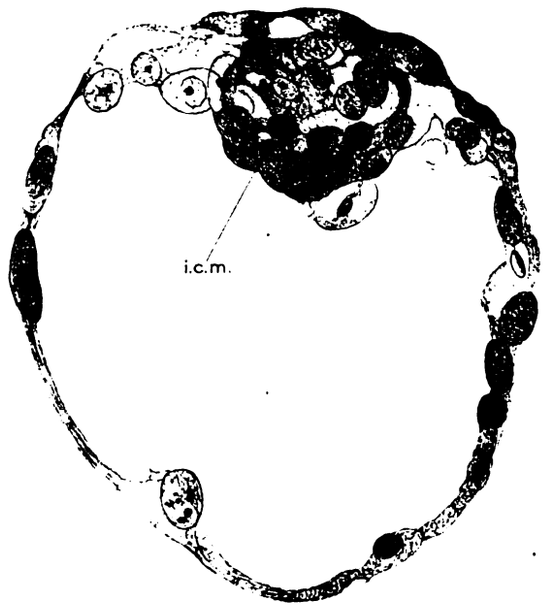

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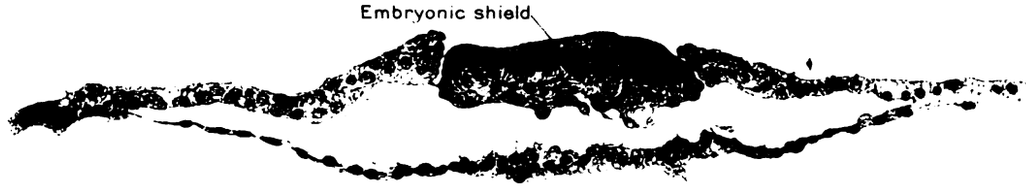

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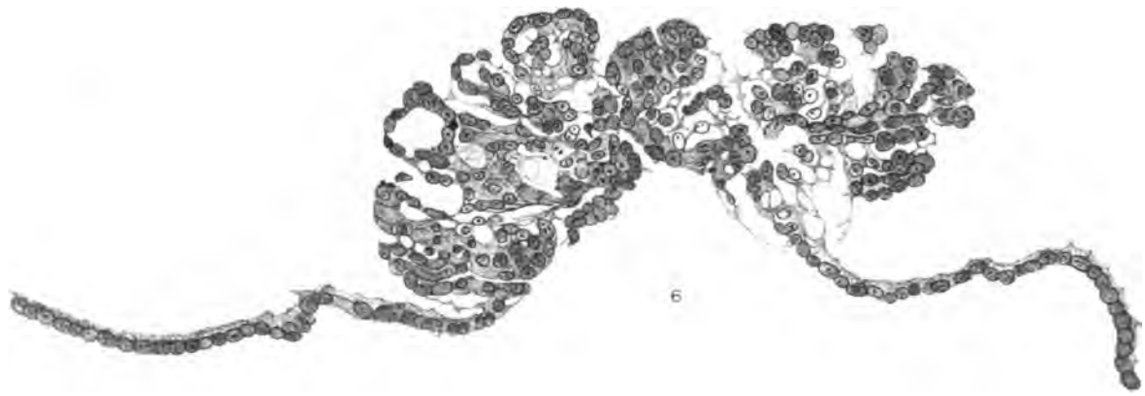

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(2) Section illustrating structure of a normal 7-day pig ovum. This is the same specimen shown in text-figure 1 a. X 600; i. c. m., inner cell mass. (3) Section of abnormal ovum shown in figure 1 b, illustrating flattening of the vesicle with partial degeneration of its cells. X 600. (4) Section of a degenerating flattened embryonic vesicle of about the 7th or 8th day. X 600. (5) Section through normal blastodermic vesicle of the 11th day. X 150. (6) Section through tumor mass in wall of early blastodermic vesicle, showing irregular growth of embryonic cells. X 150. Drawings made directly on stone.

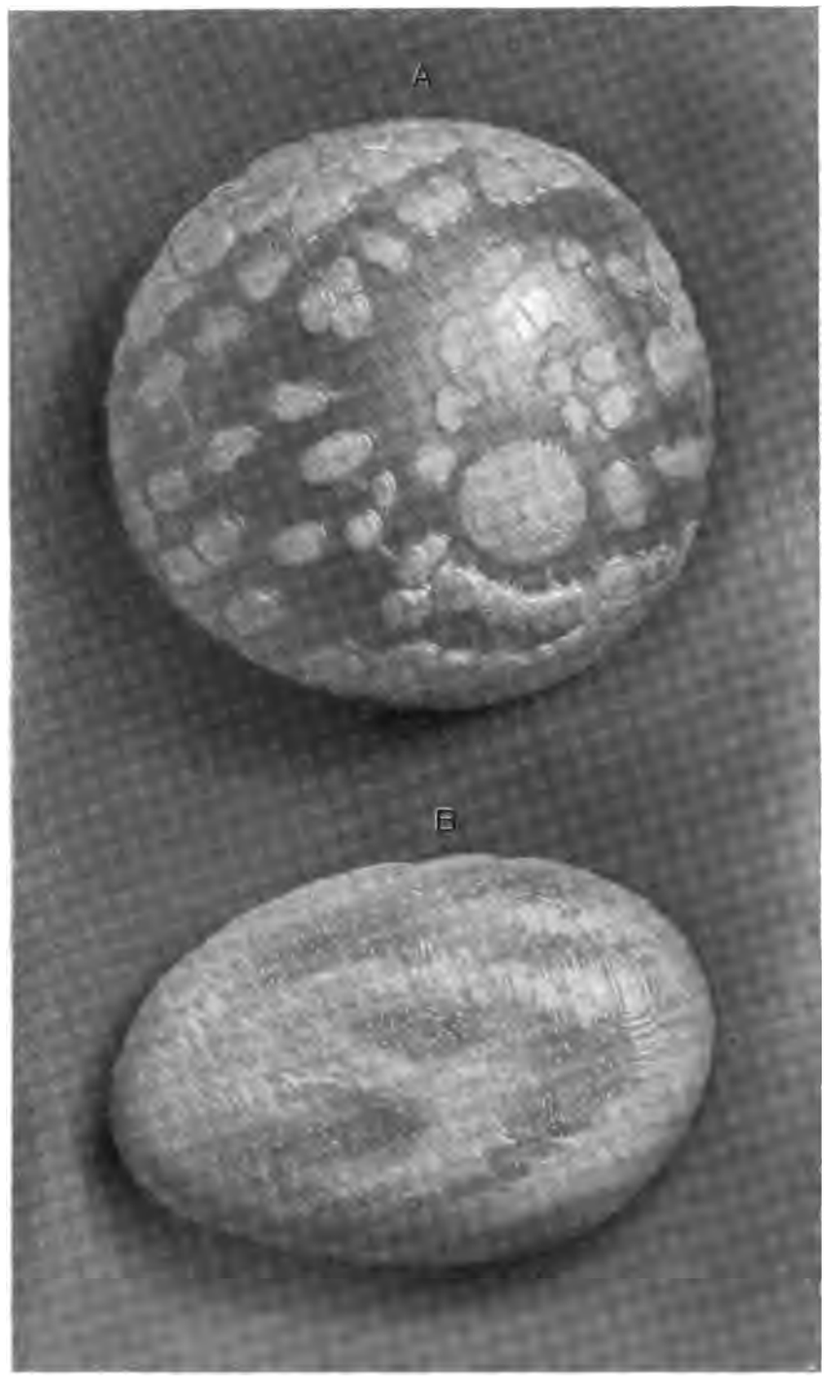

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FIG. 7.—Two blastodermic vesicles obtained from the normal uterus of a pig. One of these (A) is normal and has a development of about 7 days; the other (B) is pathological. × 600.

CONTRIBUTIONS TO EMBRYOLOGY, No. 61.

THE DEVELOPMENT OF THE EXTERNAL GENITALIA IN THE HUMAN EMBRYO.

By Milo Herrick Spaulding,
Of the University of Montana, State College of Agriculture, Bozeman.

With four plates and two text-figures.

THE DEVELOPMENT OF THE EXTERNAL GENITALIA IN THE HUMAN EMBRYO.

The present investigation is concerned with the development of the external genitalia in the human embryo, the determination of the stage at which sex differences make their appearance, and how early sex can be recognized from these external structures. In addition to the morphological interest in the structural development of the phallus region, its embryology presents several other phases of almost equal importance. Thus the ability to recognize sex at an earlier period than has hitherto been possible is of great practical value to the clinician as well as to the embryologist. At the same time, this point is largely dependent upon the verification of the existence of a definite "indifferent" ("undifferentiated," Pohlman 1904) period through which all embryos have been generally believed to pass before assuming their definite male or female characteristics.

As a result of this study I have found that there is apparently no real *indifferent* period. On the contrary, the younger embryos show constant differences in the phallus and almost from the earliest differentiation of the genital tubercle (i. e., embryos 14 mm. GL) they can be divided into two groups. This division is based upon the marked difference in the length of the urethral groove upon the caudal slope of the phallus. This seems to be quite constant and without intergradations and can be traced backward from the older embryos, in which sex can be definitely recognized, to the younger stages which heretofore have been included in the so-called "indifferent" period. In one group the urethral groove extends from the base of the phallus practically to its apex, i. e., onto the region of the future glans; these I consider to be males. In the second group the groove is much shorter and terminates some distance below the apex of the phallus—i. e., it does not extend onto the region of the future glans; these I consider to be females.

I wish to extend my sincerest thanks to Dr. G. L. Streeter for his kindness in according me the privilege of examining the extensive collection of human embryos in the Carnegie Laboratory of Embryology, as well as for the great interest he has taken and the many helpful suggestions he has made throughout the course of the investigation.

MATERIAL AND METHODS.

The material studied in the preparation of the present paper has been limited to the collection of human embryos in the Carnegie Laboratory of Embryology. This includes a large number of specimens which are graded 2 and 3; that is, they are unsuitable for sectioning because of faulty preservation, mutilation, or some other defect; also a considerable number of sectioned embryos up to 50 mm. in length. While perhaps more attention has been paid to the external features of these unsectioned embryos, as far as possible this evidence has been verified by a study of sectioned specimens of corresponding sizes, a number of these having been recon-

structed in order to correlate the development of the internal urogenital organs with that of the external genitalia.

Only a condensed tabulation of the embryos examined, with the sex classified, is included in the present report, the complete list, including the serial number of each embryo with remarks upon its condition, having been placed on file among the records of the Carnegie Laboratory of Embryology. The sex grouping adopted in this table is based upon the diagnostic features brought out by the investigation.

Table 1 shows that there is considerable variation in the relative frequency of the sexes in each of the length decades and also that there is a greater number of females than males (145 to 84) in the period between 14 and 50 mm. length. This variation in the different groups would indicate that as yet a sufficient number of embryos has not been examined to permit definite deductions concerning the sex-ratio at this period of embryonic life. Conclusions, therefore, will have to be deferred until much more extensive data have been accumulated.

TABLE 1.—*Condensed tabulation of specimens examined, arranged in length groups of 10 mm. (length decades).*

Length.	Sex.		
	Male.	Female.	Undetermined.
8 to 13 mm.	.....	.....	33
14 to 19 mm.	17	31	.....
20 to 29 mm.	41	40	.....
30 to 39 mm.	15	48	.....
40 to 49 mm.	11	26	.....
50 to 59 mm.	16	14	.....
60 to 69 mm.	25	23	.....
70 to 79 mm.	6	5	.....
80 to 89 mm.	8	3	.....
90 to 100 mm.	14	7	.....

Critical examination has been made of practically all of the embryos in the collection between the lengths of 14 and 50 mm. About 80 of these, however, have been discarded from present consideration because, owing to mutilation or poor state of preservation, a clear picture of the external genitalia could not be obtained; therefore, no interpretation of sex was possible. Of the older stages (51 to 100 mm. CR) only selected specimens have been studied. The accompanying list, therefore, includes all the well-preserved embryos between the length of 14 and 50 mm., both sectioned and unsectioned, and a small proportion of the older specimens.

No attempt was made to identify the sex of embryos smaller than 14 mm., although it is believed that a careful study of a sufficient number of such younger stages will show that the sex differences here pointed out can be traced back to the very beginnings of the genital tubercle.

HISTORICAL.

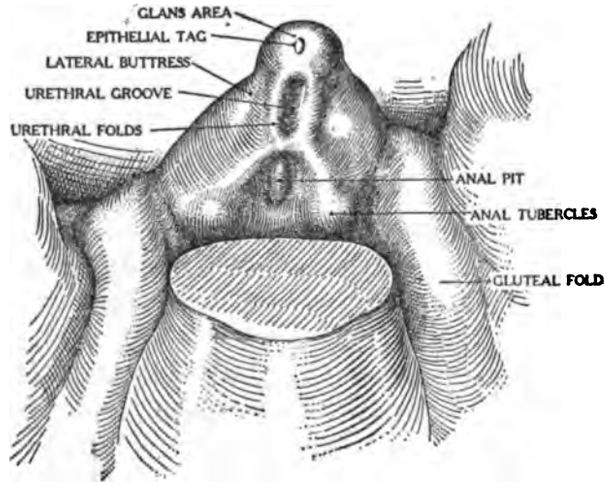
When the early scientists began the study of human embryology with the few specimens that came into their possession, the questions of sex recognition and the relations between the sexes attracted their attention to the study of the external genitalia. When we of the present day consider the paucity of their material, as well as the comparative crudity of their instruments and methods, we can not but be surprised at the accuracy of their observations and conclusions. That there were misinterpretations was due largely to insufficient material and defective apparatus, rather than to faulty observation.

As the result of this early work we find, in reviewing the literature on the subject, that various views were held by these early scientists concerning the sex of the younger embryos during the so-called *indifferent* period. Many of them considered that the younger embryos represented a stage intermediate between males and females. Others, for example Tiedemann (1813), who is conspicuous for the accuracy of his observations, believed that, because so many more females than males were observed, all human embryos were at first female and that the males resulted from an advance in development over this more primitive condition. The subsequent investigations of Joh. Müller (1830), Rathke (1832), and Bischoff (1842) resulted in but minor additions to the observations of Tiedmann. In fact, Müller considered that the latter were so remarkably complete that additional investigations could not be expected to produce anything new. Ecker (1851–59) published some excellent figures of the external genitalia of human embryos, which have served as a basis for the somewhat inaccurate Ecker-Ziegler wax models of this region. No other notable papers were published until about 1889, when the development of modern instruments, the introduction of modern methods of technique, and the consequent improvement in the quality of the preparations, resulted in a renewed interest in all branches of biological study. In the embryology of the human urogenital system this era was heralded by the work of Tourneux (1889) upon a series of 35 specimens ranging in length from 24 mm. to 35 cm. Only 10 of these were less than 40 mm. in length, while the majority were fetuses over 50 mm. in length. Although Tourneux described the external genitalia of the majority of these specimens, several of which are shown by fairly accurate figures, the number of young embryos was relatively so few that his attention was not directed toward possible sex differences in these early stages; hence, more consideration was given to the histological study of the urethral canal and the formation of the prostate. In the same year Nagel published the results of his study of a small series of human embryos. This was also devoted mainly to the internal urogenital system, although Nagel made some observations upon the external genitalia which, in part, predicted some of the results of the present study. He believed, for example, that the urethral groove should furnish a clue to the sex of an embryo at a much earlier period than it had previously been possible to recognize sex, although his observations indicated that the males possess the shorter groove, while my findings tend to show quite the opposite. Nagel furthermore found that the outer genital (labio-scrotal) swellings do not arise as a more or less complete ridge surrounding the phallus, as had previously been figured, but as separate, lateral swellings which only later become connected in front of the phallus in the female, and in the male are shifted to a caudal position to form the scrotum. The classical investigation of Keibel (1896), giving an exhaustive account of the development of the internal urogenital organs, based upon a series of 13 embryos (3 to 25 mm. in length), was probably the first study of this system by the modern method of plate reconstruction whereby it has been possible to correlate the development of the external with the internal organs. While Keibel figures the external genitalia in several of his reconstructions, especially those of embryos 11.5 and 25 mm. long,

which show the shorter urethral groove that I have found to be characteristic of female embryos, no special description is given of these structures. In fact, he realized that for the investigation of the development of the external genitalia a large series of embryos was necessary, and this portion of his paper is for the most part devoted to a plea to his colleagues for more specimens.

While I realize that I have been more fortunate than any of my predecessors in having at my disposal a larger and more complete series of embryos, I still would reiterate Keibel's plea for more material; because it seems that the more specimens one has at his disposal, the more one realizes the need of additional material in order to study some of the points concerning which there is still a conflict of opinion.

In 1904 Herzog pointed out that the direction of the phallus made it possible to recognize the sex of an embryro at about the beginning of the third month, instead of towards its close. This difference in direction he showed to consist in a greater caudal decurvation of the female phallus, while that of the male remained more nearly at right angles to the axis of the body. His series consisted of 16 embryos, varying in length from 20 to 190 mm. Of these, he figures the external genitalia of only 9, none of which bring out very clearly his point of directional difference of the phallus. Some excellent figures of the external genitalia are given by Otis (1906), although the discussion of the genitalia does not form a logical part of his paper.



TEXT-FIGURE 1.—Drawing of external genitalia of an embryo 16.8 mm. long (Specimen No. 492), illustrating the genital-tubercle period. × 25.

From 1890 to 1907 a number of valuable contributions upon the development of the urogenital system in mammalia, especially the domesticated forms, made their appearance. While a number of these (notably the series of investigations carried out by Fleischmann and his pupils) pertained more or less to the development of the external genitalia, they have proved of but little use in the present investigation, since these papers failed to bring out any early sex differences in the forms studied.

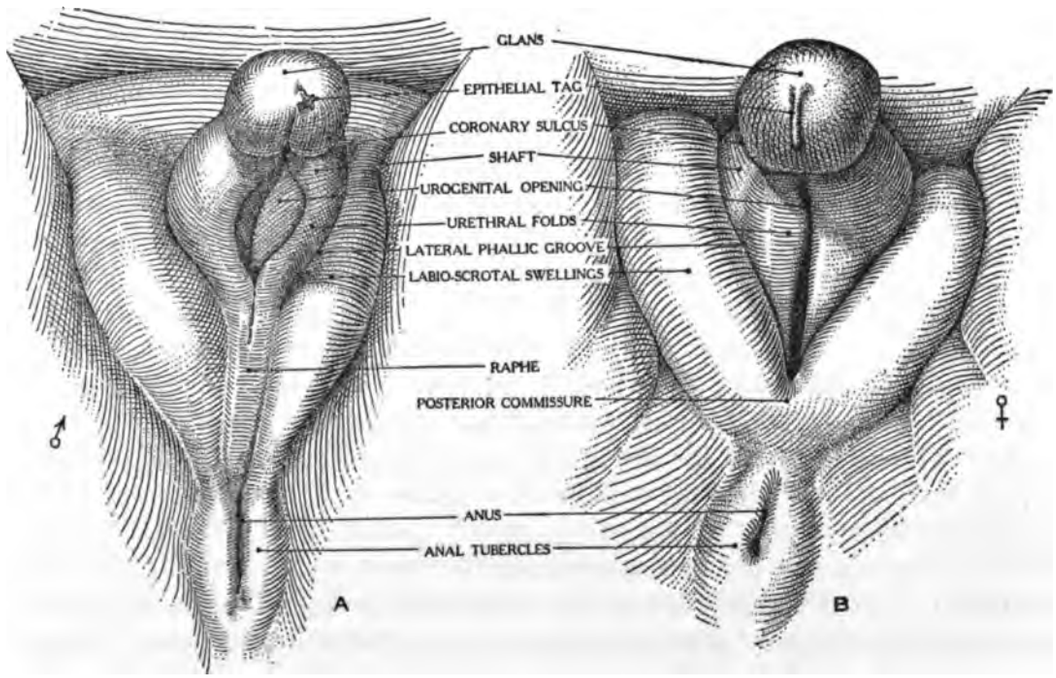
Felix (1912) gave an exhaustive summary of the accumulated observations upon the development of the human urogenital system, although his discussion of

the external genitalia is rather meager as compared to that of the internal organs. Certain of my observations have led me to conclusions different from those reached by Felix; the discussion of these moot points will be taken up in the section describing the development of the external genitalia.

STAGES OF DEVELOPMENT OF THE EXTERNAL GENITALIA.

While the greater part of the investigation has been devoted to a study of the changes occurring after the embryos have attained a length of 14 mm., a few observations have been made upon younger specimens which are here included as forming a starting-point for the present description.

For the sake of convenience, the sequence of development of the external genitalia has been divided into three periods, each of which is represented by one or more stages: (1) genital-tubercle period, characterized by the more or less conical form of the genital eminence prior to the formation of the labio-scrotal swellings; (2) phallus period, beginning with the definite appearance of the labio-scrotal swellings which separate the conico-cylindrical outgrowth of the genital tubercle from the surrounding body areas; and (3) definitive period, characterized (particularly in male embryos) by the transition of the primary external genitalia into what is essentially their final form.



TEXT-FIGURE 2.—Drawings illustrating the parts of the external genitalia at the beginning of the definitive period. *A*, male embryo 45 mm. long (Specimen No. 948); *B*, female embryo 49 mm. (Specimen No. 2256). × 20.

GENITAL-TUBERCLE PERIOD.

Stage 1, 9 mm. (fig. 1, plate 1).—The earliest stage that I have studied is shown in an embryo 9 mm. long. In this specimen the genital tubercle is a very low conical eminence between the umbilical cord and the base of the tail. Its apex is

separated from the rest of the tubercle by a pair of converging depressions which unite in the midventral line to form a longitudinal groove extending to the caudal border, giving to the entire groove a Y-shape. Caudal to the arms of the Y and lateral to its stem is a pair of broad, swollen areas. This condition is slightly different from the stage described by Keibel (1896) of the beginning development of the genitalia in a 3-mm. embryo. In his specimen he described the cloacal tubercle as consisting of a pair of eminences separated by the cloacal membrane. The difference between the stage described by Keibel and my first stage may be accounted for entirely by the difference in the size of the two specimens.

The term *genital tubercle*, which I use to describe the entire genital eminence, has a slightly different meaning from the one given to it by Felix (1912, p. 948). That author speaks of the genital eminence at first as the *cloacal tubercle*, which is later divided into a basal genital tubercle and a terminal phallus. According to his usage, the former develops into the genital (labio-scrotal) swellings, while the latter forms the phallus proper. My observations indicate that the entire primordial genital eminence should be considered as the precursor of the phallus and as such should be termed the *genital tubercle*. As will be shown later, my observations also indicate that the labio-scrotal swellings apparently originate from the outlying tissue and not from the basal portion of the primary tubercle. But even without this modified interpretation of their origin, the term *genital tubercle* seems most inapplicable to these swellings (which are decidedly secondary, both in genital structure and in function) and is therefore much more *à propos* of the genital eminence as the precursor of the phallus.

Stage 2, 8 mm. (fig. 2, plate 1).—The second stage, represented by the reconstruction model of an 8-mm. embryo, shows considerable advance in the development of the genital tubercle. This now forms a somewhat rounded mass which occupies almost the entire area between the umbilical cord and the base of the tail. Its cranial margin is but barely indicated by a slight groove between its apex and the umbilical cord. The apex is broadly rounded and from it the slightly convex caudal surface slopes toward the base of the tail. The caudal slope is almost bisected by the urethral groove, a shallow depression extending from the base to the tip of the tubercle. The basal end of the groove is separated from the anal pit by a narrow transverse bar. It is significant that, while there is this external separation of the primitive cloacal groove into the urethral groove and anal pit, the internal division of the cloacal cavity into urogenital sinus and rectum is not yet complete, at least in this embryo. The margins of the urethral groove are but slightly elevated into the urethral folds, although the margins of the anal pit are more pronounced. Laterally the caudal surface of the tubercle is rounded out into pronounced swellings. Almost in front of the tubercle the ventral body-wall bears a pair of swellings lying in the umbilico-phallic angles, the significance of which has not yet been definitely ascertained. These masses show a gradual increase in size until the embryo reaches a length of 16 mm. They then apparently disappear in some embryos, while in others they undergo a caudal shifting; this would seem to indicate that they are the primordia of the labio-scrotal swellings, which definitely make their

appearance in embryos 17 to 19 mm. long. As yet, however, a sufficient number of these younger embryos has not been examined to permit definite conclusions on this point.

Stage 3, 8 to 12 mm. (figs. 3 and 4). As growth proceeds, the genital tubercle is transformed into a compressed, conical protuberance. This is brought about by the deepening of the umbilico-phallic groove, so that the cranial slope of the tubercle is nearly straight (i. e., approximately at right angles with the body axis), while the caudal slope remains decidedly convex. At the same time the caudal outline has become markedly triangular by the broadening of the base until it occupies practically the entire area between the bases of the legs. The conspicuous lateral slopes form the "lateral buttresses" which, arising from the cranial border of the tubercle just proximal to its apical area, have a decidedly caudal trend, so that they finally disappear basally opposite the caudal border of the tubercle. The apex of the tubercle is now clearly marked off from the more proximal portion by a shallow circular depression, indicating it as the future glans and separating it from the basal shaft. The urethral groove is a long, lancet-shaped depression, broadest and deepest basally, narrowing distally into a shallow slit limited by a very small "epithelial tag" just proximal to the primitive glans area. The urethral folds (margins of the groove) are elevated as slight rolls of tissue which distally merge into the glans region, while basally they become more tumid and broaden out to surround the anal pit as anal tubercles. Lateral to these urethral folds, the caudal surface of the tubercle is somewhat swollen in the younger specimen illustrating this stage (fig. 3), in marked contrast to the decided concavity of these regions in the older embryo (fig. 4). In the former (fig. 3) the urethral and anal membranes have not ruptured. The older embryo (fig. 4) agrees with the finding that (in the majority of cases) these membranes rupture at about the stage of 12 to 13 mm., although several specimens were found, among both the sectioned and unsectioned material in which the membranes were still imperforate at 17 mm. The perforation of this membrane transforms the shallow urethral groove into a gutter-like, primitive urogenital opening as a direct communication between the phallic portion of the urogenital sinus and the exterior. This is accompanied by an increase in the definiteness of its outlines, as the result of which its sex difference in length is correspondingly emphasized. Because of the variation in the time of rupturing of this membrane, as well as on account of normal differences in the breadth of the opening thus formed, it seems advisable to continue to use the term *urethral groove*, except when referring to embryos that clearly show this feature as an opening.

While there is an apparent sex difference in the lateral outline of the tubercle in embryos 10 to 16 mm. in length, in that in some its tip is placed slightly more cranially than in others, thus producing a wider separation between the tubercle and the tail in the first group than in the second, a sufficient number of embryos has not been examined to permit of definite conclusions on this point, because of the possibility that some distortion of the embryos may have been brought about by manipulations during fixation.

Stage 4, 14 to 15 mm. (figs. 5, 7, male; figs. 6, 11, female).—In this stage the sex difference in the length of the urethral groove becomes clearly evident. But slight changes have taken place in the general shape of the genital tubercle. In the male (figs. 5, 7) the glans area is more clearly indicated than in the female (figs. 6, 11). In both embryos the urethral groove is sharply outlined, making it possible to contrast its difference in length in the two specimens. In the female the epithelial tag is more pronounced than in the male and the entire tubercle is slightly more swollen.

PHALLUS PERIOD.

Stage 5, 16 to 17 mm. (fig. 8, male; fig. 12, female).—By the time the embryo has attained a length of 16 to 17 mm. the genital tubercle has elongated into a narrow, conical organ which, because of its modified shape and its separation from the surrounding body areas by the newly formed labio-scrotal swellings, will now be called the *phallus*. The male embryo representing this stage (fig. 8) shows the phallus as more nearly cylindrical than in any of the younger embryos, the lateral buttresses having, to a marked extent, merged into its body. The groove limiting the glans is present, although not clearly shown in the photograph. The urogenital opening is a narrow orifice extending almost the full length of the phallus, limited distally by a pronounced epithelial tag. The urethral folds are considerably broader than in any of the younger stages, their outer margins being sharply separated from the remains of the lateral buttresses by a concave depression. The most pronounced change, however, is the presence of the labio-scrotal swellings as a pair of distinct, rounded ridges, one on each side of the base of the phallus and separated from it by a broad lateral phallic groove. As regards this feature, it is quite likely that this embryo represents a slightly older stage, as the male specimen of the next stage shows more clearly the probable beginnings of these swellings, and the female selected as the counterpart for this stage does not show them to any marked degree, if they are present at all. It is, of course, very possible that there is normally more or less variation in the time of development of these structures, and that this embryo (fig. 8) is merely slightly precocious in this respect.

The female of this stage is represented by the dissected-out phallus region of an embryo of the same length. The phallus has the same general configuration as that of the male just described, with the exception of a shorter urogenital opening terminated by a larger epithelial tag, a greater fullness in the genital folds, the presence of a straight postanal bar which is grooved by a slight median longitudinal depression flanked on each side by anal tubercles, and the apparent absence of the labio-scrotal swellings. The presence or absence of these swellings in this specimen must, however, remain an open question, although the indications point strongly to their absence. In this respect the embryo seems to be more nearly normal (so far as sequence in the appearance of the modified parts of the external genitalia is concerned) than its coördinate male.

Stage 6, 19 mm. CR (fig. 9, male; fig. 13, female).—In the male of this stage the phallus is still of narrow, conical form, with the glans area rather more sharply indicated by a broad, band-like depression, not as clearly shown in the photograph

as in the embryo itself. The remains of the lateral buttresses arise just proximal to this depression, spreading laterally for a short distance, then continuing basally almost parallel to the axis of the phallus until they finally merge into the tissue at its base. Unlike most of the younger embryos examined, there is almost no caudal trend to these buttresses. The urogenital opening has deepened and become more pronounced. It extends from the base of the phallus practically to its tip. In this specimen it is not limited distally by an epithelial tag. The lack of this appendage, useless as it at present seems, is probably due to some loss prior to or during fixation. The distal portion of the groove (the glans portion) forms a diamond-shaped dilatation extending to the proximal limits of the glans, the remainder of the groove being constricted into a deep, narrow slit. The urethral folds are rather pronounced elevations which diverge distally to merge into the glans, while proximally they are considerably thickened into a pair of caudally projecting, diverging masses (bulbo-urethral swellings?) which merge laterally into the outlying labio-scrotal swellings. They are separated from the cavernous portion of the phallus by broad concavities. Perhaps the most interesting feature of this embryo is that it shows the apparent beginnings of the labio-scrotal swellings. These are a pair of flat, elevated areas on either side of the base of the phallus with which they seem to be continuous. The cranial margins of these areas are nearly straight, extending at diverging angles into the inguinal regions, where their cranio-lateral angles continue for a short distance as ridges of tissue. The caudal margins appear as continuations of the diverging wings of the basal portions of the urethral folds, forming sinuous curves to the caudo-lateral angles, from which points they sweep cranially in rounding curves to the cranio-lateral angles, where they unite with the cranial margins. This is the only specimen which shows these swellings definitely connected with the base of the phallus. In all others in which they are developed there is a groove (lateral phallic groove) on each side which separates them from the base of the phallus.

As regards the formation of the labio-scrotal swellings, my observations diverge from the description given by Felix (1912, pp. 948–949):

> "The cloacal tubercle is thus divided into the almost circular base of the phallus and the semilunar *genital tubercle* (fig. 639). This latter represents an originally unpaired structure lying cranial to the phallus and surrounding it on two sides; from it there are formed later the two genital swellings. The genital tubercle is separated from the phallus in female embryos by a deep groove which is the anlage of the later sulcus nympho-labialis, the groove between the labia majora and minora. In the male embryos this groove is absent from the beginning."

My own findings indicate that the labio-scrotal swellings arise as paired outgrowths from the outlying tissue and not from a basal segment of the primary tubercle. Furthermore, all but one of the specimens I have examined, both male and female, show these swellings as separated from the phallus by the lateral phallic grooves, a condition which is in striking contrast to the concluding statement made by Felix.

The coordinate female (fig. 13) shows, more clearly than any of the younger specimens, the distinction between the glans and shaft, although the division is indicated more by changes in the surface modeling than by the formation of a definite coronary sulcus. There is also a very slight indication of the caudal decurvation which was first pointed out by Herzog (1904) as an early sex difference. The urogenital opening is clearly limited to the shaft, being broader basally and narrowing distally into a mere slit. The urethral folds are still quite broad and are separated from the cavernous portion by shallow depressions. The labio-scrotal swellings resemble those already described for the male of the preceding stage.

Stage 7, 21 to 23 mm. CR (figs. 10, 15, males; figs. 14, 19, females).—While there is a slight increase in the length of the phallus at this stage, the most conspicuous changes are the increase in the relative sex differences in the length of the urogenital opening and the greater development of the genital folds and the labio-scrotal swellings. In the males figured, the greater length of the urogenital opening is clearly seen to be in sharp contrast to the much shorter opening of the females. The urethral folds at this stage are more pronounced than in any of the embryos previously described. As a result of the deepening of the lateral depression between these folds and the cavernous portion of the shaft, their median portions now project from the shaft as compressed ridges. Distally they merge into the terminal glans area, while proximally they broaden out into the overhanging pre-anal enlargements (bulbo-urethral swellings?). As a result of this modification, the shaft of the phallus now shows clearly two distinct regions: the heavily thickened, cavernous portion, formed by the medial migration of the lateral buttresses, and the caudal urethral portion, represented by the projecting urethral folds outlining the urogenital opening, a condition which persists throughout the remainder of the phallus period. The labio-scrotal swellings have now grown into *f*-shaped enlargements, separated from the phallus by grooves more pronounced in the males than in the females. Cranially, these swellings are separated from each other by a triangular prolongation of the base of the phallus, so that there is no indication of the horseshoe shape which is so characteristic of most of the older illustrations. The caudal extremities of these swellings are joined together by a low, curving postanal ridge (slightly larger in the female), which is almost bisected by a median sagittal groove.

Stage 8, 24 to 28 mm. CR (figs. 16, 17, males; figs. 20, 21, females).—This stage is represented by an exceedingly interesting pair of twins. In the male (fig. 16) the nearly cylindrical phallus projects at approximately right angles to the body axis, in marked contrast to a pronounced decurvature of that of its mate (fig. 20). The glans is not so sharply demarked as in the female, although there are slight indications of its extent. The urogenital opening extends almost to the tip of the phallus as a shallow, open groove deepened basally by the caudally extended, plate-like urethral folds. As a result of this increase in the folds, the base of the phallus is considerably longer than its apex. The labio-scrotal swellings are separated from the base of the phallus by broad, shallow grooves; their tips curve cranio-laterally away from the phallus, leaving a rather broad, unswollen area between the latter and the umbilical cord.

In the female (fig. 20) the glans has become more clearly defined, in a negative way, by changes which have taken place in the character of the urethral folds. As a result of this modification the entire shaft is terminally decurved upon the sharply demarked urethral folds, which now apparently form plate-like supports for the thicker cavernous shaft, the terminal portion of which (the future glans) is slightly more knob-like than that of the male. The inclosed urogenital opening is narrow and lanceolate, clearly restricted to the region of the genital folds and not extending onto the decurved glans. In general, the labio-scrotal swellings are similar to those of the male; their caudal prolongation into a curving, connecting postanal ridge is, however, more pronounced. Here it is without tubercular enlargements, but is almost bisected by a median groove.

Some of the older embryos in this stage show further changes of a minor nature in the phallic region. Thus the glans may be more strongly indicated by a further increase in the density of its tissue (corpus cavernosum glandis), so that it appears as an opaque white tip in sharp contrast to the more translucent shaft. The postanal ridge, joining the tips of the labio-scrotal swellings, becomes more V-shaped, while the inclosed anal opening shifts from a transverse to a longitudinal slit.

Reconstruction models of three embryos of this stage show quite clearly the correlation between the sex differences of the external and internal organs. In one specimen (No. 584*a*, 25 mm. CR) showing externally the long male type of urogenital opening, the tip of which extends onto the glans, the internal organs are male also. The gonad is rather short, barely reaching to the rim of the pelvis, while the tubar portion of the urogenital folds, containing the Wolffian and Müllerian ducts, unite with the dorsal wall of the bladder without the formation of a genital cord (primordium of the uterus).

In the females modeled (No. 840, 24.8 mm. CR, and No. 782, 28 mm.) the opposite condition is found. Externally the phallus of each shows a short urogenital opening. Internally the organs are decidedly more of the female type. The gonads are slightly longer, while the tubar portions of the urogenital folds (containing the Müllerian ducts) unite to form a genital cord before fusing with the dorsal wall of the bladder, thus producing a sinuous ridge which projects from the dorsal wall of the bladder and partially subdivides the cavity of the pelvis. In the older specimen the gonads are relatively larger and more compact.

Stage 9, 30 to 38, mm CR (figs. 18 to 23, males; figs. 22 and 29, females).—The changes in form between this and the previous stage are slight and consist mainly of a further decurvation of the female phallus and a great condensation of the glans area, although as yet the definite coronary sulcus has not been formed. In the male, the urogenital opening still extends the entire length of the phallus and there is no indication of the raphé.

DEFINITIVE PERIOD.

Stage 10, 38 to 45 mm. CR (figs. 24 to 28, 31, 32, males; figs. 30, 35, 36, females).—The series of important changes occurring between the lengths of 38 and 45 mm. culminates in the final differentiation of the external genitalia. The forma-

tion of an open coronary sulcus demarks the terminal, knob-like glans from the remainder of the phallus. The most significant changes, however, are those involved in the separation of the urogenital opening and anus. These modifications, more extensive in the male than in the female, are brought about by the transformation of the open, gutter-like urogenital sinus into the tubular urethra, due to the almost complete fusion of the urethral folds to form the raphé. At the same time the labio-scrotal swellings migrate from their primitive lateral position to a new one caudal to the penis, forming the scrotum.

An early stage in this series of modifications is shown in figure 24. The ridge-like raphé now separates the anal opening from the proximal end of the lenticular urogenital opening, the distal end of which continues to the tip of the glans as a narrowing slit. Coincident with this fusion of the urethral folds to form the raphé, the labio-scrotal swellings shift slightly, so that their greatest tumidity now lies caudal to the penis instead of lateral to it, as heretofore.

Regarding the formation of the scrotum, Felix (1912) makes such conflicting statements that it is not at all clear just what his conclusions are. In his preliminary account (p. 953) he states:

"The basal growth of the pars pelvina must produce a new area, interposed between the base of the penis and the anal opening, and the best name for this is the *unpaired scrotal area* (fig. 642)."

The figure referred to by him, according to my interpretation, is that of a female specimen, and the area he labels as the unpaired scrotal area I should designate as the posterior commissure. He continues:

"In embryos of 60 mm. head-foot length this (unpaired scrotal area) becomes raised up *in toto* and forms the unpaired scrotal swelling, into which the two genital swellings, which we may now term the scrotal swellings, extend from above . . . . As soon as the descensus is complete this unpaired scrotal area alone forms the scrotal sack, the paired scrotal swellings to the right and left of the penis fading out into the surrounding areas."

Later, in referring to the development of the labia majora in the female, he says (p. 955):

"The two genital swellings on this account are not prolonged anally to form an unpaired scrotal tubercle, and consequently they do not fade out as in the male but persist as the labia majora."

Finally, in his resumé of the development of the external genitalia he concludes (p. 958):

"In the male the tuberculum genitale fades out in all its parts and in its place there is formed at the anal periphery of the phallus, from the unpaired scrotal area, a new swelling, the unpaired scrotal sack."

An analysis of the above shows that he has made two statements implying that the labio-scrotal swellings are transformed into the scrotum (*in toto* or in part), and two other equally emphatic statements implying that they play no part in its formation. Furthermore, if we accept his first statement, it is still doubtful

whether he considers that there is a caudal migration of the labio-scrotal swellings into the scrotum, or whether the final formation of the latter is accomplished by an anal prolongation of these swellings with an accompanying cranial atrophy.

Subsequent growth results in the gradual reduction of the broad urogenital opening into a small aperture of varying size and shape, located just proximal to the coronary sulcus. The reduction in the size of this opening is usually accompanied by the development of a more or less pronounced epithelial rim and an increase in the size of the epithelial tag into a comb-like ridge of varying size and shape. This sequence of events is so clearly shown in figures 24 to 28, 31, and 32 that no detailed description of the individual steps is necessary. Attention should be called, however, to the ridge-like development of the penial raphé in the embryo illustrated in figure 27, where it forms a penial-scrotal frenulum, binding the penis to the scrotum with a consequent decurvation. The production of this frenulum is quite characteristic for most of the older embryos, although as a rule it does not become noticeably developed until after the embryos have attained a length of 55 mm.

The changes taking place in the female at this time are not so extensive. As in the male, a definite coronary sulcus is formed. The other modifications may be considered as a faint paralleling of the more profound metamorphosis which the male genitalia have been undergoing and consist chiefly of changes in the labio-scrotal swellings. The caudal ends of these draw toward each other (fig. 35) and finally fuse to form the posterior commissure (figs. 37, 38), definitely separating the urogenital opening from the anus, although this is slight in comparison to the corresponding separation in the male. In this manner, the labio-scrotal swellings are transformed from separate, lateral swellings into a cranially open, horseshoe-shaped rim partially encircling the phallus. Unlike the corresponding change in the male, this fusion is not accompanied by either a caudal migration of these swellings or by the formation of a definite raphé.

Regarding the formation of the posterior commissure, my observations are decidedly at variance with the conclusions drawn by Wood-Jones (1913) that in the adult (human) female this is not definitely present as a commissural bar separating the vaginal orifice and anus, but that it merely shows as such when the genitalia are placed in an unnatural position.

The posterior commissure may be considered as marking the advent of the definitive period in the female, and from now on we may refer to the component parts of the external genitalia by their adult terms. The labio-scrotal swellings form the labia majora, connected by the posterior commissure. The cavernous portion of the phallus becomes the clitoris, divided into glans and shaft. The urethral folds constitute the labia minora, margining the persisting primary urogenital opening (the urethro-vaginal orifice). It must be pointed out, however, that the use of these latter terms at this time is an arbitrary one, because the division of the female phallus into clitoris and labia minora is never entirely complete and is consummated only with the formation of the frenulum clitoridis at some time after the close of the period covered by the present investigation (100 mm. CR length).

For this reason, the strict usage of these terms would require the continued use of the inclusive term *phallus* until the separation is culminated.

The phallus period is thus terminated by the formation of the raphé and the scrotum in the male, and the posterior commissure of the labia majora in the female. Thus the definitive characteristics of the external genitalia are organized and the recognition of the sex of the embryos is correspondingly simplified. These observations, in the strict sense, diverge from the statement made by Felix (1912, p. 949):

> "The beginning of the sexual differentiation can hardly be assigned to a definite period, since it takes place quite gradually and even in advanced stages presents difficulties to diagnosis. We base our diagnosis on the position of the ostium urogenitale relative to the coronary sulcus on the one hand and the anal opening on the other. Male embryos are distinguished by the distal (*distal* here has reference to the base of the phallus) circumference of the ostium urogenitale always lying in the coronary sulcus, while the proximal circumference becomes more and more distant from the anal opening. Female embryos may be recognized by the urogenital opening retreating away from the coronary sulcus by the gradual closure of its distal part, while its proximal part always lies close to the anal opening; the differentiating moment is, accordingly, both positive and negative in each sex."

While this statement, if it be interpreted broadly, may to a certain extent be considered as containing the germ of the diagnostic point which I have found to be of value in the early recognition of sex in the human embryo, it plainly does not agree with my own observations, in that Felix limits it to the final period when I find definitive changes taking place in the external genitalia, and at which time the modifications are so characteristic that examination of the external genitalia almost at once enables one to definitely say that one embryo is male and another is female. It must be emphasized, however, that it is still necessary to make careful examination of the genitalia to obtain a correct diagnosis, because from this time throughout the rest of the fetal period individual variations are more pronounced than in the preceding embryonic period. In many of the females, for example, the clitoris attains considerable size, and if this alone is considered, or if only a cursory examination is made, such an embryo might easily be mistaken for a male. (A comparison of figs. 33 and 34, males, with figs. 37 and 46, females, will illustrate this point.) In fact, in the majority of males between the lengths of 50 and 100 mm. CR, the exposed penis seems to be comparatively shorter than the clitoris in females of the same size, an effect which is largely produced by the varying development of the penial-scrotal frenulum.

So few pronounced morphological changes take place in the developing genitalia during the rest of the period covered in this study that it will not be necessary to divide them into successive stages.

Although I have not yet been able to make a complete study of the development of the prepuce, my observations upon its formation from the external examination of older embryos may be of value and are therefore included in the present account as suggesting the apparent formation of this structure, although

the final corroboration of this will have to be left for future investigation. For this reason, it seems inadvisable at this time to enter into any comparison of the various explanations that have been made to account for its origin.

After the separation of the phallus into shaft and glans by the formation of a coronary sulcus, the glans remains as a naked, knob-like termination of the shaft (in both sexes) until the embryo reaches a length of about 65 mm. CR. In males of this size the first traces of the prepuce may be noted. The skin of the shaft becomes elevated into a pair of conspicuous rolls on either side of the urethral opening (fig. 41). Gradually these rolls join on the dorsal side of the shaft (fig. 42) to form a flat ridge whose distal margin has grown out to cover the proximal edge (corona) of the glans (embryos 75 mm. CR). The continued outgrowth of this fold results in the gradual inclosure of the glans (figs. 47 to 50) until (embryos 98 to 100 mm. CR) the glans is completely covered by the prepuce.

Accompanying the development of the prepuce, there is a gradual shifting of the urethral opening until (in embryos 85 to 90 mm. CR) it occupies a subterminal position in the frenular notch of the glans. With the completion of the prepuce this primary opening is entirely closed. Some time later a new (the secondary or definitive) terminal urethral opening is formed near the tip of the glans.

But few important changes take place in the female genitalia in this later period (50 to 100 mm. CR length), these being mainly concerned with the continued growth of the labia majora and the beginnings of prepuce formation. The labia majora increase somewhat in height, so that the inclosed structures become more submerged in the rim which they form, although this submergence is by no means completed at this period.

The formation of the prepuce is considerably more involved than in the male and all of the folds included in its complete development do not make their appearance until some time after the close of the period under consideration. The only observations made are concerned with the glandular portion. This fold apparently develops in much the same way as it does in the male, although it has not been studied as closely and is not as well shown in the figures. Its growth is by no means as rapid as in the male, with the result that the glans of the clitoris is not completely covered by it at the close of this period (100 mm. CR). As has already been noted, the complete separation between the clitoris proper and the labia minora does not take place until considerably later.

SUMMARY.

A more comprehensive idea of the sequence of events in the development of the external genitalia may be gained if we briefly review their development in each sex.

DEVELOPMENT OF THE MALE GENITALIA.

The genital tubercle, from which is derived the phallus and which includes the urethral and anal openings, arises as a broad conical eminence barely separated cranially from the umbilical cord, but with a well-defined caudal slope. Its rounded free end, as the primordium of the glans, is faintly separated from the basal portion. The lateral slopes, below the glans area, extend into the outlying basal tissue as the "lateral buttresses," which I interpret as enlargements for the developing corpora cavernosa. The caudal slope is bisected by the shallow urethral groove whose margins are slightly elevated into the urethral folds. Distally, these margins merge into the glans area, while proximally they continue into the basal enlargements surrounding the anal pit. In the majority of embryos which have attained a length of 12 mm. the urethral membrane is ruptured to form the primitive urogenital opening, although a few cases have been found in which this perforation had not taken place at the stage of 16 to 17 mm. Practically from its first appearance the urethral groove shows a sex difference in its length, this difference being further accentuated upon the formation of the urogenital opening.

As development proceeds, the elongation of the tubercle and the medial migration of its lateral buttresses transform it into the somewhat cylindrical phallus, whose base is separated from the surrounding body areas by the newly formed labio-scrotal swellings (embryos 17 to 19 mm.). The latter, appearing first as broad, elevated areas extending laterally from the base of the phallus, are soon transformed into swollen ridges separated, in both sexes, from the base of the phallus by the lateral phallic grooves. It may be emphasized that in the majority of specimens the labio-scrotal swellings seem to be from the first separated from the phallus. In fact, only one embryo was found which showed these areas as definitely merging into the phallus, while in all of the others of about the same age the swellings were already separated from the phallus by pronounced grooves, the lateral phallic grooves.

Accompanying the elongation of the tubercle into the phallus, the urogenital opening becomes gradually more sharply outlined and the sex difference in its length correspondingly emphasized. The urethral folds which form the margins of the opening also increase in definiteness, partly through their own elevation and partly through their lateral demarcation from the cavernous portion of the shaft; the result of these combined factors being that the folds extend as plate-like caudal projections from the more cylindrical shaft. At the same time the terminal glans has increased in density so that it now forms an opaque white area in contrast to the translucent shaft, although the limiting coronary sulcus is not formed until the close of the phallus period.

As a result of these combined changes the male phallus at this time is markedly different from that of the female. The length of the urogenital opening is still,

however, the chief diagnostic feature in the two sexes. In the male it consists of a long slit extending from the base to the apex of the phallus, whereas in the female it extends only to the base of the glans. In the male it is more open distally, the apposition of the urethral folds reducing it proximally to a mere slit. This is in marked contrast to the condition in the female, where the opening (restricted to the shaft) has exactly the opposite shape, being broadest basally, while the terminal portion is narrowed into a slit.

No further pronounced morphological changes take place in the male genitalia until after the embryo has reached a CR length of 38 mm. At this time a series of changes begins which result in the transformation of the male genitalia into approximately their final form (about 45 mm.) and in the complete separation of the urogenital opening from the anus. The gutter-like urogenital sinus is transformed into the tubular urethra by the merging of the basal portions of the urethral folds into the raphé, reducing the primitive urogenital orifice to an irregularly shaped opening on the distal portion of the shaft. At the same time the glans becomes sharply defined by the formation of a wide coronary sulcus and the labio-scrotal swellings assume their final position caudal to the penis to form the scrotum, the halves of which become more or less closely approximated to the raphé in the mid-ventral line, although they never entirely lose their bilateral character.

This period (45 mm. CR length) marks the completion of the definite sex differentiation, characterized by the development of definite structural features in the male in sharp contrast to the lack of these characters in the female.

As growth continues, the gradual constriction of the urethral opening synchronous with the formation of the prepuce, to be described later, is accompanied by an outgrowth of its rim which, in embryos 60 to 85 mm. CR length, results in the formation of a decided cup in the bottom of which the opening is located. With the later stages of prepuce formation the urethral opening is shifted to a more terminal position in the frenular notch of the glans. It then becomes entirely closed and eventually there is formed the new, permanent terminal urethral opening on the apex of the glans.

The first evidence of the prepuce is found in embryos of about 65 mm. CR length, at which time it can be recognized as a pair of swellings on each side of the urethral opening. Gradually these swellings fuse together over the dorsum of the shaft to form a flattened ridge of skin whose distal margin has enveloped the proximal margin (corona glandis) of the glans (embryos 75 mm. CR length). Subsequent growth results in the progressive inclosure of the glans by this distally migrating fold of skin until at about 100 mm. CR length the originally naked glans is entirely covered. It must be emphasized that these observations upon the formation of the prepuce are based only upon the external examination of the genitalia of these older embryos and have not been confirmed by histological study. For this reason they must be considered merely as suggestions of what apparently takes place and may later be corroborated or contradicted when it becomes possible to make a study of sectioned embryos showing this development.

DEVELOPMENT OF FEMALE GENITALIA.

The entire process of development of the external genitalia in the female is accompanied by fewer pronounced morphological changes than occur in the male. It is noteworthy, however, that (in spite of this greater simplicity in structure) completion of development is more protracted, so that the final differentiation of the female genitalia, although brought about by comparatively minor changes, does not synchronize with the more complete transformation of the male (45 mm. CR length), but instead, beginning with a slight change at a length of about 50 mm., extends as a gradual process throughout a considerable period of early fetal life.

From its beginning until the stage of 21 to 25 mm. the genital tubercle of the female closely resembles that of the male except for the shorter urethral groove. About this period the female shows the beginnings of the caudal decurvation, which is apparently brought about by an excess in the growth of the cavernous over the urethral regions of the phallus. At the same time the urethral folds have become compressed into plate-like caudal projections supporting the slightly overhanging glans, which in this way is more clearly defined than in the male. As has already been pointed out, the coronary sulcus is not formed in either sex until the embryos reach a length of about 45 mm. From 25 mm. to 45 mm. this caudal decurvation becomes a more and more pronounced characteristic of the female phallus, and for this reason is a diagnostic feature of increasing importance as development proceeds.

In the female important changes occur at about the stage of 45 to 50 mm. CR length, and these likewise mark the termination of the phallus period. While much less extensive than the correlated changes in the male, they are nevertheless characteristic and indicate the approximate assumption of the final form. The most pronounced modification occurring at this time is that the caudal ends of the labio-scrotal swellings grow towards each other and finally join in the midventral line to form the posterior commissure (50 mm. CR length). In this manner these originally paired swellings are transformed into a cranially open, horseshoe-shaped rim, inclosing the rest of the external genitalia and separating them from the anus.

The formation of the posterior commissure in the female thus synchronizes with the formation of the raphé in the male and may be considered as representing the advent of the final differentiation, and from now on we may refer to the genitalia by their adult terms. The labio-scrotal swellings form the labia majora. The glans and cavernous portion of the phallus may be considered as the clitoris, and the urethral folds as the labia minora. The inclosed primary urogenital opening may now be called the urethro-vaginal orifice. It must be pointed out, however, that the application of these terms at this time is an arbitrary one, because the actual separation of the female phallus into these more definitely adult structures does not take place until some time after the close of the period included in the present study (100 mm. CR). Strict accuracy would demand that, until such division had been completed by the formation of the frenula clitoridis, the inclusive term *phallus* be retained.

Because of the persistence of the urethral folds (labia minora) in the female and their failure to fuse together as they do in the male, the female phallus retains a more conical outline than does the penis of the male.

In female embryos of 60 to 100 mm. CR length there is shown a partial development of the prepuce, although the complicated set of folds involved in its complete formation is not produced at this time and only partial growth of the glandular portion is completed at the close of this period. While the glandular prepuce is apparently formed in much the same way as it is in the male, its growth is markedly slower, and in embryos 100 mm. long the glans is not completely surrounded by it.

In these older embryos (60 to 100 mm. CR length) there is also some increase in the height of the labia majora, so that the inclosed portions are somewhat submerged in the rim thus formed, although this submergence is by no means as complete as it becomes in later fetal life. It should also be noted that in fetuses up to 100 mm. CR the labia majora are still cranially separated and there is no indication that they play any part in the formation of the mons veneris.

CONCLUSIONS.

1. There is a morphological sex difference in the external genitalia of the human embryo practically from their first appearance, characterized by marked difference in the length of the urethral groove upon the caudal slope of the genital tubercle. In some embryos the distal end of the groove extends onto the glans region (males), while in others it does not reach to this region (females).

2. This should prove to be a fairly reliable character for the recognition of sex in human embryos at an earlier period than has heretofore been possible.

3. From the first appearance of the genital tubercle, there apparently is no *indifferent* or *undifferentiated* period in the development of the external genitalia before they acquire their definite male or female characteristics.

4. The caudal decurvation of the female phallus, first pointed out by Herzog, is a valid diagnostic characteristic and is applicable at an earlier period than was first indicated.

5. The phallus period is definitely terminated in both sexes by the assumption of approximately the adult form of the genitalia. In the male this is brought about by the fusion of the urethral folds into the raphé and the accompanying caudal migration of the labio-scrotal swellings to form the scrotum. In the female it is characterized by the fusion of the caudal ends of the labio-scrotal swellings to form the posterior commissure.

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IX. Die äusseren Genitalien des Schafes (Böhm), vol. 34, 248–320.
X. Die Teilung der Kloake bei Cavia cobaya (Dimpfl), vol. 35, 17–64.
XI. Das Analrohr des Schafes (Schwartstrauber), vol. 35, 65–74.
XII. Bau und Entwicklung der äusseren Genitalien bei Cavia cobaya (Gruber), vol. 36, 1–26.
XIII. Die äusseren Genitalien des Schweines (Dürbeck), vol. 36, 517–543.
XIV. Der äusseren Genitalien der Hauskatse (Dürbeck), vol. 36, 544–565.
XV. Tabellarische Übersicht der Genitalentwicklung bei Säugetieren (Dürbeck), vol. 36, 566–569.
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J. F. Didusch *fecit.*

FIG. 1.—No. 467, 9 mm. × 21.
FIG. 2.—No. 792, 8 mm.
FIG. 3.—No. 2702, 8 mm. × 18.5.
FIG. 4.—No. 1784*a*, 12 mm. × 21.
FIG. 5.—No. 1936, 14 mm., male. × 14.5.
FIG. 6.—No. 2023, 15 mm., female. × 14.5.

NOTE: Figures 1, 3, 4, 5, and 6 are drawings made directly from the specimen. Figure 2 was drawn from the model. The remaining figures are photographs; some of these, owing to difficulty in obtaining clear negatives, have been retouched to bring out certain details of structure that would otherwise be lost.

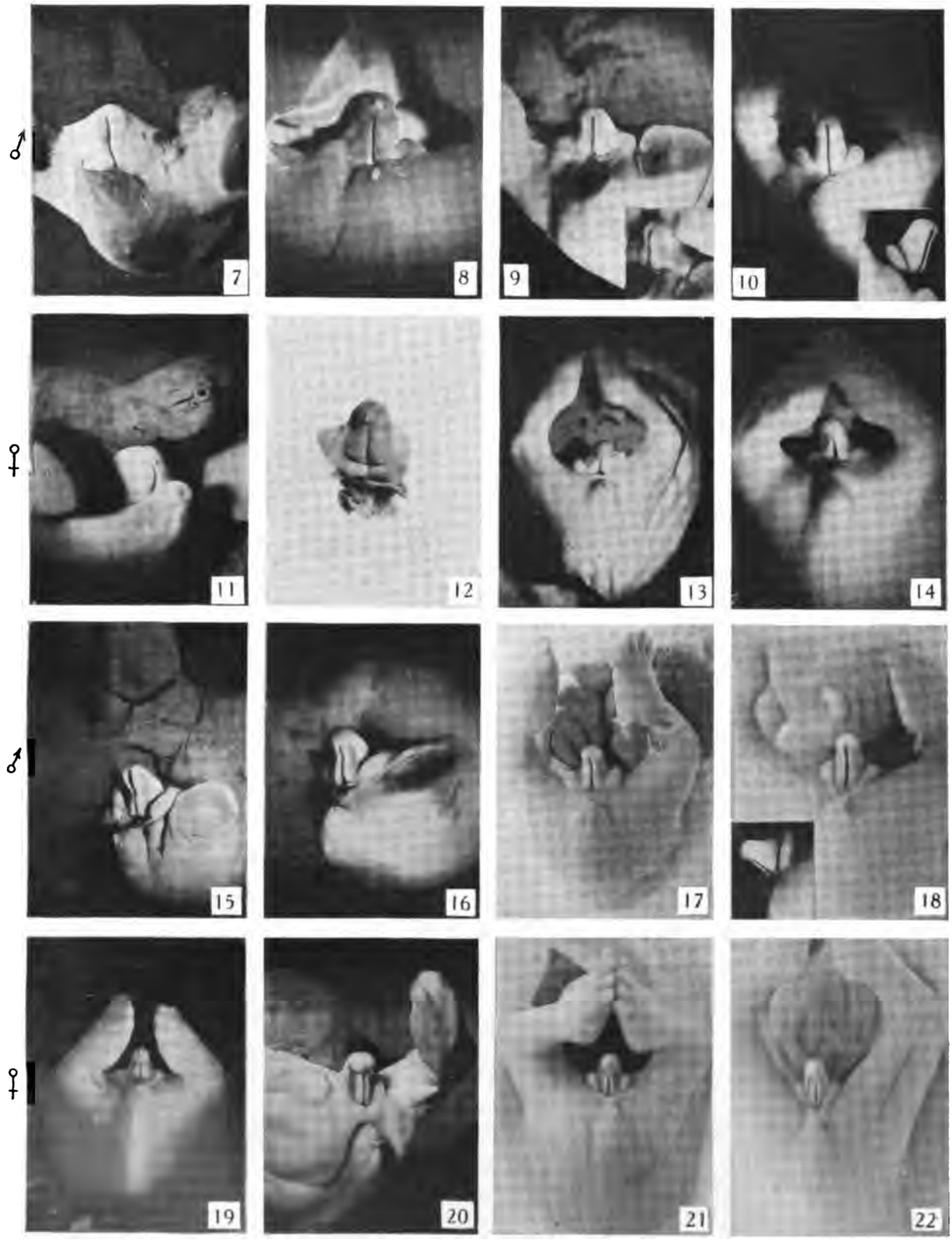

FIG. 7.—No. 1936, 14 mm., male. × 12.
FIG. 8.—No. 955, 17 mm., male. × 12.
FIG. 9.—No. 28, 19 mm., male. × 6. (Insert, lateral view.)
FIG. 10.—No. 389*a*, 21 mm., male. × 6. (Insert, lateral view.)
FIG. 11.—No. 2023, 15 mm., female. × 12.
FIG. 12.—No. 1750, 17.2 mm., female. × 12.
FIG. 13.—No. 684, 20 mm., female. × 6.
FIG. 14.—No. 194, 21 mm., female. × 6.
FIG. 15.—No. 590, 23 mm., male. × 6.
FIG. 16.—No. 1900–60*b*, 25 mm., male. × 6.
FIG. 17.—No. 879*c*, 29.6 mm., male. × 6.
FIG. 18.—No. 1022*d*, 32 mm., male. × 6. (Insert, lateral view.)
FIG. 19.—No. 2393, 23 mm., female. × 6.
FIG. 20.—No. 1900 60*a*, 25 mm., female. × 6.
FIG. 21.—No. 950, 29 mm., female. × 6.
FIG. 22.—No. 1358*b*, 33 mm., female. × 6.

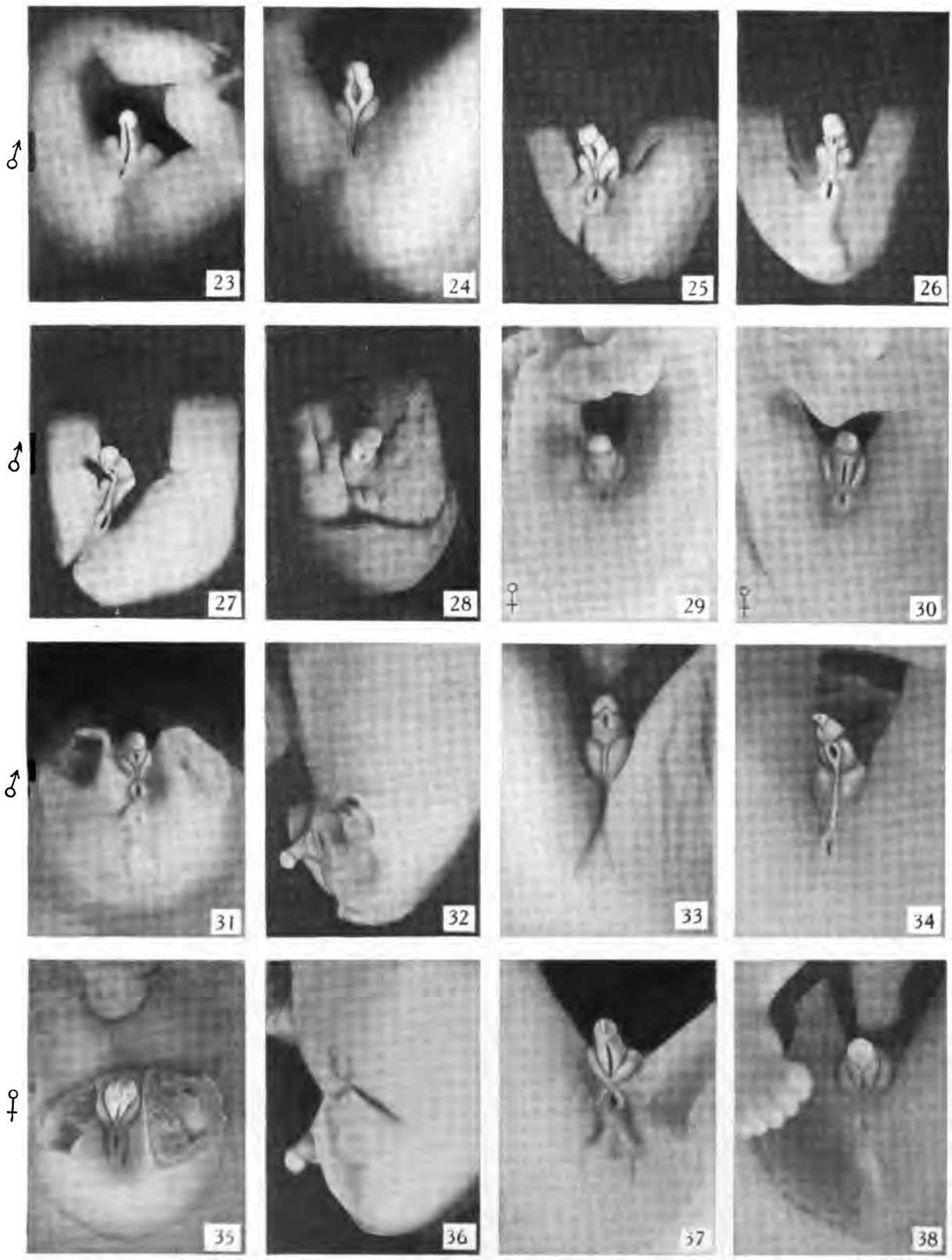

FIG. 23.—No. 1206, 37 mm., male. × 4.
FIG. 24.—No. 948, 45 mm., male. × 4.
FIG. 25.—No. 652*k*, 44 mm., male. × 4.
FIG. 26.—No. 217, 45 mm., male. × 4.
FIG. 27.—No. 693, 45 mm., male. × 4.
FIG. 28.—No. 607, 38 mm., male. × 4.
FIG. 29.—No. 2250*b*, 36 mm., female. × 4.
FIG. 30.—No. 2250*a*, 40 mm., female. × 4.
FIG. 31.—No. 1686, 46 mm., male (perineal view). × 4.
FIG. 32.—Same (lateral view). × 4.
FIG. 33.—No. 1474*d*, 58 mm., male. × 4.
FIG. 34.—No. 847, 58.8 mm., male. × 4.
FIG. 35.—No. 1597*b*, 44 mm., female (perineal view). × 4.
FIG. 36.—Same (lateral view). × 4.
FIG. 37.—No. 1388, 51 mm., female. × 4.
FIG. 38.— No. 1625*b*, 54 mm., female. × 4.

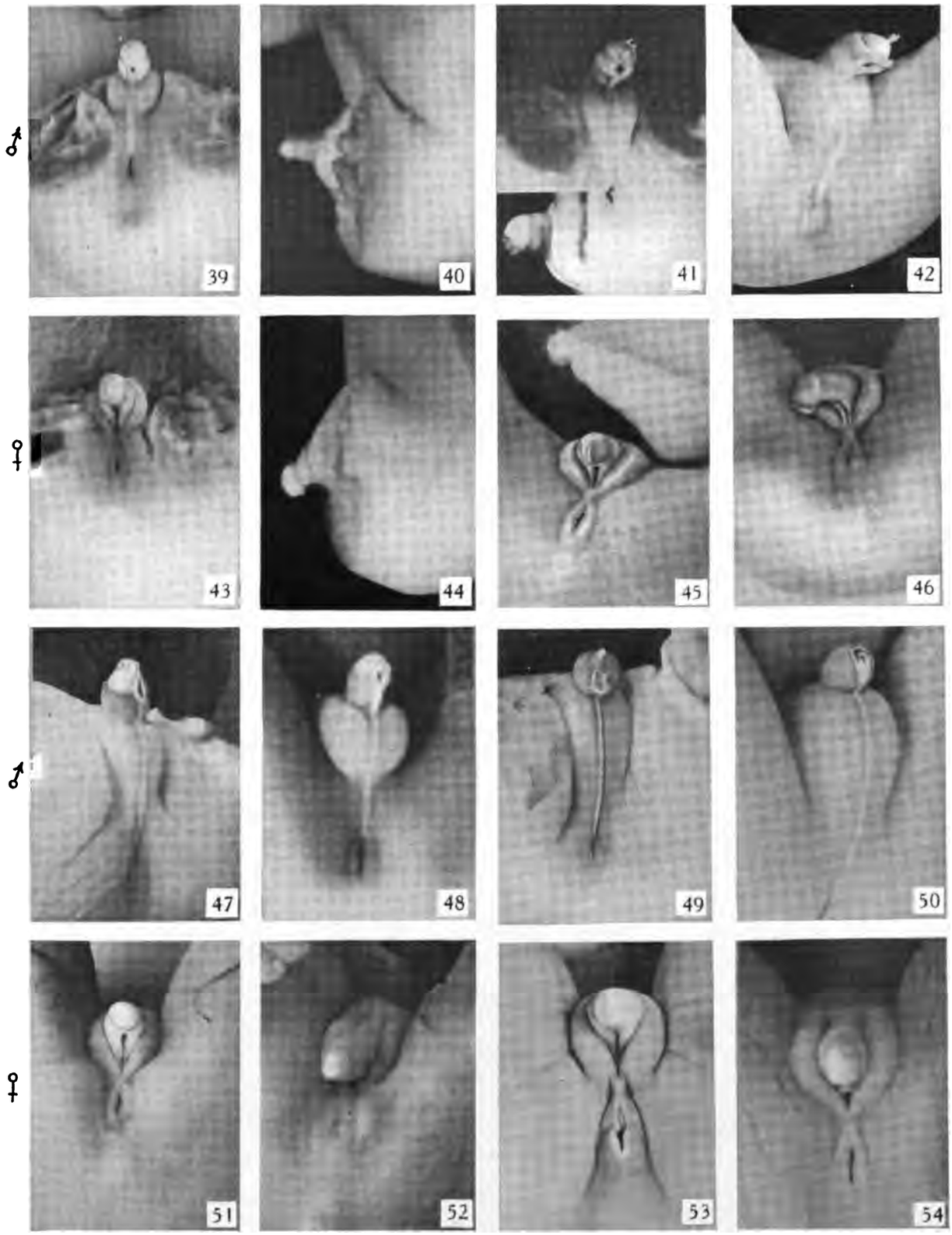

FIG. 39.—No. 1183, 60 mm., male (perineal view). × 4.
FIG. 40.—Same (lateral view). × 4.
FIG. 41.—No. 1163, 68 mm., male. × 4. (Insert, lateral view).
FIG. 42.—No. nn34*k*, 75 mm., male. × 4.
FIG. 43.—No. 907, 60 mm., female (perineal view). × 4.
FIG. 44.—Same (lateral view). × 4.
FIG. 45.—No. 1282*b*, 65 mm., female. × 4.
FIG. 46.—No. 1542, 69 mm., female. × 4.
FIG. 47.—No. 2026, 80–90 mm. (est.), male. × 4.
FIG. 48.—No. 1705*a*, 83.2 mm., male. × 4.
FIG. 49.—No. 1852, 95 mm., male. × 3.
FIG. 50.—No. 834, 98 mm., male. × 3.
FIG. 51.—No. 1455, 78 mm., female. × 4.
FIG. 52.—No. 1474*b*, 84 mm., female. × 4.
FIG. 53.—No. 1831, 93.5 mm., female. × 4.
FIG. 54.—No. 1476, 100 mm., female. × 4.

CONTRIBUTIONS TO EMBRYOLOGY, No. 62.

FURTHER EXPERIMENTAL STUDIES ON FETAL ABSORPTION.

III. THE BEHAVIOR OF THE FETAL MEMBRANES AND PLACENTA OF THE GUINEA-PIG TOWARD TRYPAN BLUE INJECTED INTO THE MATERNAL BLOOD-STREAM.

IV. THE BEHAVIOR OF THE PLACENTA AND FETAL MEMBRANES OF THE RABBIT TOWARD TRYPAN BLUE INJECTED INTO THE MATERNAL BLOOD-STREAM.

By George B. Wislocki,

Of the Anatomical Laboratory, Johns Hopkins Medical School.

With one plate.

FURTHER EXPERIMENTAL STUDIES ON FETAL ABSORPTION.

III. THE BEHAVIOR OF THE FETAL MEMBRANES AND PLACENTA OF THE GUINEA-PIG TOWARD TRYPAN BLUE INJECTED INTO THE MATERNAL BLOOD-STREAM.

Our knowledge of the behavior of vital dyes in the pregnant animal rests upon the careful observations of Goldman (1909) upon mice and rats. He observed that when vital dyes, such as trypan blue and pyrrhol blue, were injected into the maternal circulation, they were not transmitted through the placenta to the fetus. The barriers which prevent the dye from entering the fetus are the chorionic ectoderm of the placenta and the epithelium of the vitelline membrane. In the rat and mouse the latter constitutes the outermost fetal covering. He also noted the interesting fact that the placenta acts as an attraction center for vital dyes, since, with the initiation of pregnancy in an animal which has been vitally stained, the dye is to a great extent given up by the liver, spleen, and other tissues in which it is stored and is conveyed by the blood-stream to the developing placenta, where it is immediately absorbed by the chorionic epithelium and the endodermal cells of the vitelline membrane.

The present studies, which are a continuation of a series of investigations on fetal absorption (Wislocki, 1920), concern the behavior of the placenta and fetal membranes of the guinea-pig and rabbit toward trypan blue. Although similar in many respects to the observations of Goldmann on the rat and mouse, there are differences sufficiently great to give interest to a description of the process of vital staining in these rodents.

The guinea-pigs used in these experiments were stained by the technique usually employed in administering vital dyes. There was no opportunity to study the placenta and fetal membranes in the early stages of their development, and the following description applies only to the period from the time the fetus reaches a length of about 15 mm. up to term.

In an animal killed at the end of a week after six injections of dye-stuff the maternal tissues were found to be deeply stained. Each fetus was completely surrounded by its vitelline membrane, which was stained dark blue, the color being deepest in a villous zone adjacent to the placenta and fading somewhat towards the antimesometral pole. The amniotic fluid was usually colorless but sometimes showed a slight tinge in deeply stained animals. The amnion, when stripped away from the vitelline membrane and examined in salt solution, as a rule was seen to be uncolored; in rare instances, however, it showed a blue tint, attributable to large and repeated injections of the dye. The fetus and umbilical cord likewise were usually entirely unstained, although a barely perceptible blue coloration of the fetus was noted in several instances, associated with a bluish tinge in the amniotic fluid and membrane.

Goldmann noticed in the mouse and rat the staining of the amniotic membrane and fluid, but never any of the fetus. He believed that the dye in the amnion must

be derived from the neighboring vitelline membrane and this, he thought, was an argument in favor of the origin of the amniotic fluid, in part at least, from the maternal blood. It is more probable that the trypan blue occasionally seen in the amniotic fluid of rodents first enters the fetal blood-stream through both the vitelline membrane and the placenta. A trace of the dye in the fetal blood might readily escape detection, whereas, as soon as it had diffused from there into the colorless amniotic fluid it would attract attention. That it immediately enters the amnion from the vitelline membrane, without first entering the fetal circulation, seems improbable, for the vitelline membrane is well vascularized by the omphalomesenteric vessels and, should a dye diffuse from its epithelium through the basement membrane, it would necessarily be swept into these large vascular channels before it could enter the amnion.

The outside of the placenta appeared blue and on gross section the entire organ was found similarly stained with the exception of an area about 5 by 3 mm., which occupied a central position between the placental labyrinth and decidua (fig. 1). As will be shown later, this white tissue consists of chorionic villi possessing a peculiar arrangement and designated by Duval as "the roof of the central excavation."

In many rodents, such as the rat, mouse, and guinea-pig, the vitelline membrane, as described by Duval and Sobotta, becomes the outermost fetal covering and persists throughout intrauterine life. Its epithelial surface comes in direct contact with the uterine wall and serves to nourish the fetus by the absorption of embryotrophe and the assimilation of extravasated maternal blood. This is more readily accomplished by the development of numerous villi which project into the uterine space and are covered by a single layer of cylindrical, phagocytic epithelial cells. This layer stains very rapidly and deeply with trypan blue (figs. 1 and 2). A single injection suffices, after a few hours, to cause the cytoplasm of the cells to become heavily laden with fine blue granules. One is justified in speaking of the cells covering the villi as an attraction center for vital dyes, for nowhere else in the body are the dye-stuffs so rapidly taken up.

The villi show their greatest development near the line of attachment to the placenta, while towards the equator of the vitelline membrane they become progressively lower. Finally they disappear, so that the membrane at its antimesometral pole is perfectly smooth. During this transition the epithelium changes from a cylindrical to a cuboidal type. The latter is probably less phagocytic than the former, for the cuboidal cells contain relatively few trypan-blue granules.

The villi cease suddenly near the attachment of the vitelline membrane to the placental surface (figs. 1, 2), and the cylindrical, vitally staining epithelium changes abruptly to a delicate, flattened type which fails to absorb the dye. This unstained, flattened tissue completely lines the angle (sinus entodermaticus) formed by the membrane at its attachment to the placenta. As the vitelline membrane (ectoplacental endoderm) spreads out laterally over the surface of the placenta, its epithelium again undergoes a change (fig. 2). The cells proliferate, forming in many places small tufts or clusters, the older cells of which gradually become constricted off by new cells arising from the basement membrane.

Duval noted the remarkable papillary hyperplasia of the epithelium in this region but did not ascribe to it any function. These cells do not stain vitally, as do those of the neighboring villi, and therefore their function possibly is not one of phagocytosis or absorption. It is interesting to note that the greatest proliferation of the epithelium occurs during the middle of pregnancy and that towards term it ceases. At birth the vitelline membrane in this region has been reduced to a single layer of columnar or cuboidal epithelial cells resting upon a simple basement membrane.

We shall now consider the vitally stained placenta. From a knowledge of the behavior of the placenta of the mouse and rat towards trypan blue, we should expect to find the dye abundantly present in the chorionic epithelium. We should look for it particularly in the giant cells and syncytium of fetal origin which invade and destroy the maternal tissue and attach the placenta to the uterine wall, and also in the delicate epithelial syncytium of the labyrinth which covers the villi and forms a barrier between the maternal blood-stream and the fetal capillaries.

To the unaided eye the placenta appears quite blue, with the exception of a tiny flattened area near its center, which always remains unstained (figs. 1, 4). On microscopic examination one is surprised to find very little of the dye stored within the chorionic epithelium. If the animal has not been deeply stained one may fail to find any at all. In a well-stained guinea-pig, it can be distinguished as a fine blue tracery in the chorionic epithelium of the placental labyrinth (fig. 3). The stain is faint, however, as compared with its exhibition in the same cells of the mouse and rat.

In the giant cells and syncytium of fetal origin which invade the decidua and form an irregular boundary between the fetal and maternal tissues, commonly referred to as the central zone or "Umlagerungszone," no trypan-blue granules are found. This is surprising, since in the injected mouse and rat these cells are filled with blue pigment throughout gestation. Possibly the fetal syncytium stains vitally in the guinea-pig in younger stages, when the ectoplacental cone is sending out syncytial roots and is burrowing its way into the decidua. However, in the youngest fetus (15 mm.) observed in these experiments no dye was found, nor was any found in the syncytium which, in the guinea-pig, divides the placental labyrinth into numerous lobules, the so-called *interlobular* syncytium.

We have already mentioned that the roof of the central excavation, a structure peculiar to the placenta of the guinea-pig, stands out in the gross as an unstained area. When the mesoderm containing allantoic vessels invades the ectoplacenta, the latter is virtually hollowed out and a central core of mesodermal tissue is formed. This has been termed by Duval the *central excavation*. Part of this mesoderm finally invades the central zone, hollows out the masses of trophoblast situated there, spreads out over a broad area, and sends into the decidua a number of villous processes covered by several layers of ectodermal cells. This conspicuous structure, the function of which is unknown, has been designated by Duval as the *roof of the central excavation*. It persists throughout gestation but the decidual tissue which it embraces between its slender, finger-like processes gradually degenerates and, as term approaches, becomes converted into a homogeneous substance. The villi

themselves, together with the vessels supplying them, appear to degenerate to a great extent. It is interesting to note that this same area is conspicuously blue when the vital dye is injected into the fetal instead of into the maternal blood-stream (figs. 4, 5).

The behavior of the surface of the placenta, just beneath the vitelline membrane, is also noteworthy. The proliferation of the epithelium of the ectoplacental endoderm has already been described and need not be referred to further. Very early in the development of the placenta a layer of fetal syncytium comes to lie directly beneath the membrane. The periphery of this syncytium, however, very soon undergoes a change. It shapes itself into two or three layers of ectodermal cells with large round nuclei surrounded by a wide zone of vacuolated cytoplasm. Duval called attention to these cells on the surface of the placenta of the guinea-pig and referred to them as giant cells. In a section through the border of the placenta, at about the middle of pregnancy, one sees the vitelline membrane beneath which are layers of these giant cells, beneath which in turn is a layer of border syncytium containing large lacunæ, and finally the placental labyrinth (fig. 6). In an animal which has been stained with trypan blue the ectoplasm of the giant cells is filled with fine granules of the dye (fig. 6). As the end of gestation approaches, these cells become shrunken and in part disintegrate and disappear.

In the decidua serotina groups of free macrophages, containing varying amounts of trypan blue in their cytoplasm, were encountered. Many of these wandering cells appeared to be undergoing degeneration. They were similar to those described by Goldmann in the mouse and rat, which were thought by him to be important in the transfer of nutrient material, such as glycogen, from the tissues and vessels of the decidua to the fetal ectoderm. In the myometrium and serosa, in the neighborhood of the placental attachment, hundreds of similarly stained cells were observed. Goldmann believed that in the mouse and rat many of these are derivatives of the serous cells covering the peritoneal surface of the uterus. This may also be the case in the guinea-pig, for the peritoneal cells were conspicuously loaded with the dye.

One further aspect of the behavior of trypan blue is worthy of description. In several of the guinea-pigs used in these experiments large injections of trypan blue were administered, resulting in mild inflammatory changes in the placenta, to judge from a poor staining reaction on the part of the syncytium and the presence of numerous polymorphonuclear leucocytes in the sinusoids. It was observed that the polymorphonuclear leucocytes in the maternal blood-spaces contained numerous tiny granules of trypan blue within their cytoplasm (fig. 7). It has been the general belief that none of the elements of the circulating blood normally stain with vital dyes. Downey (1917), however, has attempted to show that the polymorphonuclear leucocyte is capable of ingesting vital dye under certain conditions. In the circulating blood conditions are unfavorable, he claims, for phagocytosis of any kind to occur, and he bases his argument on the failure of the polymorphonuclear leucocytes, while moving rapidly, to take up bacteria, vital dye, or any other particulate matter. Downey has shown experimentally that in the areas where the

velocity of the blood-stream is diminished, or outside of the blood-vessels, the leucocytes stain with vital dyes, and he believes that this staining is quite comparable to that seen in macrophages. The question is still debatable whether or not his assumption is correct and whether the staining should be considered as physiological rather than as an indication of cell injury.

The placentæ in which the staining of the polymorphonuclear leucocytes was observed showed evidence of slight inflammatory changes, but there was nothing in the appearance of the leucocytes themselves to condemn them as moribund. Further evidence is necessary, however, before it can be decided definitely whether or not true vital staining of polymorphonuclear leucocytes ever occurs.

In conclusion, a few observations upon the fate of a fine suspension of carbon particles, such as one finds in india ink, injected into the blood-stream of the pregnant guinea-pig, will be described. Several animals were injected and killed on the second, fourth, and sixth day thereafter, respectively. In these animals the liver and spleen appeared very black while the color of the other abdominal organs was almost normal. On opening the thorax the lungs were seen to contain considerable carbon. The bone-marrow was also conspicuously black, and traces of carbon were visible to the unaided eye in many other tissues, such as the ovaries, pancreas, omentum, diaphragm, and mesentery. In the uterus, placenta, or vitelline membrane, however, no carbon was recognizable. The fetuses appeared perfectly normal, the amniotic fluid clear and colorless. Under the microscope particles of carbon were found in great abundance in the liver, spleen, and lungs, where they had been phagocytosed by endothelial and connective-tissue cells. In the omentum, mesentery, bone-marrow, and diaphragm, similar but less numerous particles were encountered; in the placenta and fetal membranes, on the other hand, there was not even a trace of carbon.

In order to determine whether the cells of the vitelline membrane refuse under all circumstances to take up carbon particles, a small quantity of ink was injected directly into the uterine cavity in the region of the villi. Microscopic examination of the villi after this experiment showed that the cells covering them did not take up a single particle of carbon into their cytoplasm, although they were seen actively engaged in the absorption of hemoglobin. It appears, therefore, that the endodermal cells of the vitelline membrane and the chorionic epithelium of the placental labyrinth are unable to phagocytize particles as coarse as those of india ink, although they are capable of absorbing a substance as finely dispersed as trypan blue.

SUMMARY.

1. Trypan blue, injected into the pregnant guinea-pig, stains the placenta and the vitelline membrane but does not enter the amniotic fluid or the fetus, except in traces.

2. A zone of the vitelline membrane covered by villi absorbs the dye very rapidly and in large amount, and may well be termed an attraction center for the dye. The portion of the vitelline membrane covering the placenta, the ectoplacental endoderm, fails to stain vitally.

3. Trypan blue is absorbed and stored to a slight extent by the ectoderm covering the chorionic villi of the placental labyrinth. It is not taken up at all by the interlobular syncytium or by that part of the ectoderm, composed of giant cells and syncytium, which occupies the central zone (Umlagerungszone).

4. The dye appears in the form of fine granules in the layer of giant cells described by Duval, situated beneath the ectoplacental endoderm.

5. It is also taken up by a host of free macrophages which are found throughout the decidua serotina and the uterine wall.

6. Carbon particles injected into the maternal blood-stream are not phagocytosed by any cells of the placenta or fetal membranes.

IV. BEHAVIOR OF THE PLACENTA AND FETAL MEMBRANES OF THE RABBIT TOWARD TRYPAN BLUE INJECTED INTO THE MATERNAL BLOOD-STREAM.

Successive doses of trypan blue were administered intravenously to six pregnant rabbits, after which the animals were killed. Gestation was more than half completed at the time the last injection was done, and therefore no observations were made during the early stages of development.

On opening the abdomen the usual distribution of color was observed. The conspicuous uterus, which ordinarily contained from 6 to 12 fetuses, was deeply stained, both on surface view and on section. The placentæ were all dark blue. The vitelline, or outermost fetal membrane, was dark blue, the color being deepest in an equatorial zone between the mesometral and antimesometral poles of the fetal sac. The stain faded gradually toward the antimesometral pole, while in the opposite direction it ceased abruptly in the region of the terminal sinus. A band of unstained membrane—the chorion læve—consequently remained between the terminal sinus and the border of the placenta.

The vitelline membrane, loosely attached to the amniotic membrane by strands of mesoderm, was easily stripped, revealing the delicate amnion inclosing the amniotic fluid. The amnion itself was faintly stained and the amniotic fluid contained traces of the dye.

When large and repeated doses of trypan blue are injected into the maternal blood-stream, the amniotic fluid may become quite deeply stained. In such cases the fetus also shows a light staining. In the less deeply stained animals, in which the amniotic fluid contains only a trace of dye, a hardly appreciable staining of the fetus may be observed. Such a staining as this might easily escape detection. It is the writer's belief that the fetus probably contains at all times as much trypan blue as the amniotic fluid, but that the dye, when present only in traces, is less readily visible in the fetal tissues than in the amniotic fluid. The escape of traces of dye from the maternal into the embryonic circulation and the amniotic fluid appears to impair in no way the vitality of the fetus.

The observation that soluble dye-stuffs of various kinds, injected into the maternal blood-stream, can be detected frequently in traces in the amniotic fluid but less often in the fetal tissues, has led a number of investigators to conclude that fluid diffuses directly from the maternal blood-stream into the amniotic fluid, and that it is not necessary for the substances to enter first the fetal circulation; nor are they supposed to reach the amniotic fluid through the placenta, but are thought to enter it directly through the fetal membranes. One theory uses these observations to prove that the amniotic fluid is in whole or in part a transudate from the maternal vessels.

Leaving the human being out of consideration, the question of the possibility in animals (from observations on which the claim is based) of the formation of the amniotic fluid in this way meets with several grave objections. None of the observers working on rodents have taken into consideration the fact that the amniotic sac is completely surrounded by the vitelline membrane. This membrane possesses an unbroken epithelial surface whose cells are concerned chiefly in the

transfer of products from the embryotrophe to the fetal circulation. Furthermore, it is richly vascularized and consequently the amniotic fluid is completely surrounded by fetal blood-vessels which would make it difficult to conceive of dye-stuffs diffusing through the membrane without first entering the fetal circulation. Moreover, from the present observations and those on the guinea-pig recorded in the study just preceding, it appears probable that dyes such as trypan blue, when administered in large doses, stain the fetus as well as the amniotic fluid. The portal of entry of these traces of dye could be either the placenta or the vitelline membrane, or both, but it appears likely that, whichever route the dye pursues, it first enters the fetal blood-stream.

In the human being it is theoretically conceivable that fluid may diffuse directly from the maternal blood-stream into the amniotic fluid. The wall of the amniotic cavity contains no fetal vessels and the chorion is intimately fused with the uterine mucosa in which prominent maternal vessels are visible. If fluid escapes from these vessels it would seem quite possible that some of it might diffuse through the amniotic membrane directly into the amniotic sac.

It is of interest to consider the differences which exist in various animals in regard to the staining of the amniotic fluid after injection of a vital dye into the maternal blood-stream. Goldmann (1909) first noted the staining of the amniotic fluid in the rat and mouse after the administration of vital dyes. The author has observed the passage of traces of trypan blue into the amniotic fluid of the guinea-pig and rabbit. In similar experiments on cats (Wislocki, 1920), however, the amniotic fluid, as well as the allantoic fluid, remained unstained. The conclusion to be drawn from these observations is that the fetal membranes and placentæ of rodents are slightly permeable to ultra-microscopic particles, such as trypan blue, while those of carnivorous animals are impermeable.

Römer (1905) made parallel observations upon the permeability of the mem branes and placenta in different classes of animals. He injected tetanus antitoxin into pregnant sheep, cows, rabbits, guinea-pigs, and human beings. He observed that the antitoxin passed readily from mother to fetus in the human, that it was transmitted only occasionally in the guinea-pig and rabbit, and that transmission never occurred in sheep or cows. What is the explanation of this striking difference in behavior? Römer concluded from his experiments that the more heterogeneous the substance the more readily is it transmitted by the placenta. Thus tetanus antitoxin, which was derived from the horse, was extremely heterogeneous for man but only slightly so for the cow and sheep. Another explanation suggests itself, both for Römer's results and for the passage of traces of trypan blue through some placentæ and not through others. We must recall that Grosser (1909) has classified placentæ according to the degree of union which exists between the fetal and maternal tissues. Thus, in the pig, cow, sheep, etc., the simplest types, there occurs merely an apposition of the chorionic ectoderm to the unbroken surface of the uterus. In carnivora, such as the cat and dog, which represent the next highest type, the chorionic ectoderm has invaded the uterine mucosa so that the placenta is made up of maternal blood-vessels intimately surrounded by fetal tissue. In rodents and

man, the highest types, a still more intimate union of fetal and maternal tissues occurs. Here the maternal blood eventually flows in channels completely lined by fetal ectoderm, and hence only a layer of syncytium and delicate connective tissue separates the maternal blood from the fetal capillaries. It is apparent that in these animals (rabbit, guinea-pig, mouse, rat, and man) in which tetanus antitoxin or traces of trypan blue have been found in the fetus or the amniotic fluid, an extremely thin barrier of cells separates the maternal from the fetal blood-stream. In the cat, sheep, and cow, on the other hand, where the union is less intimate and numerous cells intervene between the two circulations, the transmission of antitoxin and trypan blue has not been observed.

The vitelline membrane of the rabbit is composed of a single layer of columnar cells, resting on a basement membrane beneath which are numerous blood-vessels. The surface of the membrane at its antimesometral pole is smooth, while in the neighborhood of the terminal sinus, where it ends in a ragged edge, it is covered by villi which project into the semi-fluid embryotrophe imprisoned between them and the uterine wall. In the injected animal the vitelline membrane is deep blue on gross appearance, and microscopically granules of trypan blue are found in large numbers in the columnar cells composing the membrane (fig. 8). These large granules, usually 20 or more to a cell, are scattered throughout the cytoplasm. The single oval or round nucleus contains none of the dye. No granules are visible in the basement membrane or beneath it in the walls of the fetal vessels. The amniotic membrane contains no trace of dye.

In the rabbit the labyrinth comprises the bulk of the placenta during the second half of pregnancy. Septa of fetal connective tissue, containing branches of the umbilical vessels, divide it roughly into lobules. The umbilical vessels break up within the lobules into capillaries which are completely lined by endothelial cells and are accompanied by delicate supporting strands of mesoderm. The space between the capillaries is occupied by a syncytium composed of fetal ectoderm, the meshes of which surround innumerable tiny spaces in which the maternal blood circulates. In consequence of this arrangement the maternal and fetal blood-streams are separated by only a thin layer of syncytium and the delicate endothelium of the fetal capillaries. In fact, in many places even the syncytium appears to be absent, so that the maternal blood-cells come in direct contact with the fetal endothelium, an arrangement no doubt greatly facilitating the interchange of substances.

Trypan blue was found abundantly present in the placental labyrinth as aggregations of tiny granules throughout the syncytium (fig. 9). None of it was seen in the cells forming the fetal capillaries. Beneath the labyrinth, and uniting it to the uterine wall, lies a layer of giant cells. These cells, which early in gestation play a prominent part in the growth of the placenta and the nutrition of the fetus, are reduced during the latter half of gestation to a relatively inconspicuous layer. In these vitally stained rabbits the cytoplasm of these cells became filled with numerous tiny blue granules, showing that the cells, even with approaching maturity, possess the power to assimilate materials brought to them.

Beneath the layer of giant cells lies the outermost or decidual layer of the placenta, composed of decidual cells, many of which were found to be undergoing degeneration. This layer is traversed by conspicuous maternal vessels which pass to and from the labyrinth. Trypan blue was present only in the cells which were degenerating and whose cytoplasm and nuclei, as a result, stained diffusely. The uterine musculature was noteworthy because of the number of vitally stained macrophages visible in its connective-tissue septa.

SUMMARY.

1. Trypan blue injected into the maternal blood-stream of the pregnant rabbit stains the placenta and vitelline membrane.

2. The dye passes in traces from the maternal into the fetal circulation, staining the fetus and amniotic fluid very slightly.

3. The dye is stored in the form of granules in the cells of the vitelline membrane and in the syncytium and giant cells of fetal origin in the placenta.

CONCLUSIONS.

Our knowledge concerning the behavior of the placenta and fetal membranes toward colloidal dyes injected into the blood-stream may be summed up as follows:

Finely dispersed colloids, such as trypan blue and pyrrhol blue, when injected intravenously into the pregnant animal, reach the placenta and are there absorbed and stored in the form of granules in the chorionic ectoderm. In the mouse and rat the dye passes in traces into the amniotic fluid but fails to stain the fetus (Goldmann). In the guinea-pig and rabbit it likewise passes in traces to the amniotic fluid, but in addition it faintly stains the fetus. In the cat vital dyes are not transmitted, even in traces, to the amniotic fluid or to the fetus (Wislocki, 1920). This variation in behavior may be explained on comparative anatomical grounds, differences of architecture making the placenta of carnivora less permeable than that of rodentia. Vital dyes are also absorbed and concentrated into granules in the cytoplasm of the cells of the vitelline membrane, which in rodentia forms the outermost fetal covering. Granules of dye are deposited in a structure peculiar to carnivora, termed in the cat the "brown border," which is a modified portion of the chorionic membrane bordering the placenta.

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DESCRIPTION OF PLATE.

ch. ep., Chorionic epithelium.
d. s., Decidua serotina.
ec. endo., Ectoplacental endoderm.
endo. v., Endodermal villi.
g. c., Giant cells.
p. l., Placental labyrinth.
r. c. e., Roof of the central excavation.
s. endo., Sinus endodermaticus.
v. m., Vitelline membrane.

FIG. 1. Gross specimen showing appearance of the vitelline membrane and placenta of a fetal guinea-pig, measuring 18 mm. after injection of trypan blue into the maternal blood-stream. The deeply stained villous portion of the vitelline membrane is readily distinguishable, as is also the unstained roof of the central excavation.

FIG. 2. Section through vitelline membrane of guinea-pig at its attachment to the surface of placenta. The character of the epithelium covering the various portions of the vitelline membrane is clearly shown. The endodermal cells clothing the villi are loaded with trypan-blue granules. The curious papillary hyperplasia of the ectoplacental endoderm is well illustrated.

FIG. 3. Section through placental labyrinth of guinea-pig, showing tracery of trypan blue in the chorionic epithelium after repeated administration of the dye.

FIG. 4. Specimen showing distribution of trypan blue in the placenta of guinea-pig after injection of the dye into maternal blood-stream. Note that the only unstained area is the roof of the central excavation.

FIG. 5. Specimen showing the distribution of trypan blue in the placenta of guinea-pig, after injection of the dye into the fetal circulation. Note that when the dye reaches the placenta through the fetal vessels, the roof of the central excavation becomes stained, but none of the dye diffuses into the decidua.

FIG. 6. Section through the surface of the placenta of guinea-pig (about the middle of pregnancy) showing the vitally stained giant cells beneath the ectoplacental endoderm.

FIG. 7. Section of the placental labyrinth of guinea-pig showing polymorphonuclear leucocytes containing granules of trypan blue.

FIG. 8. Section through the vitelline membrane of the rabbit, showing the columnar cells covering the villi filled with granules of trypan blue.

FIG. 9. Section from labyrinth of rabbit's placenta, showing syncytium in which numerous tiny granules of trypan blue are visible.

WISLOCKI

PLATE 1

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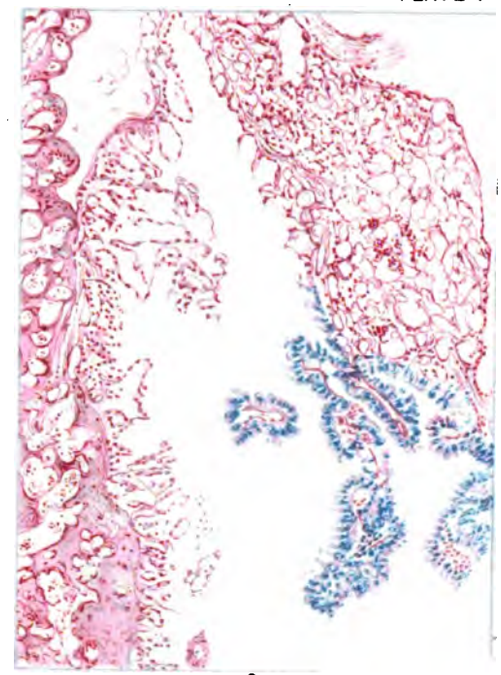

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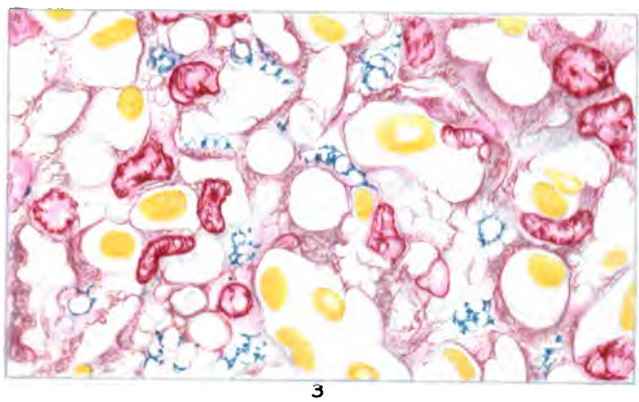

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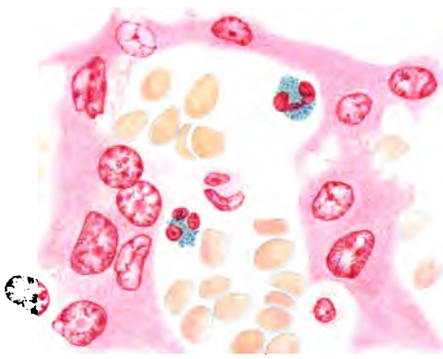

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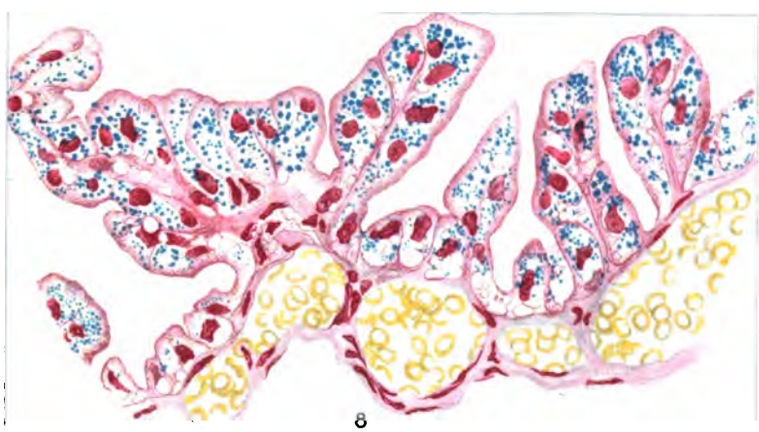

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CONTRIBUTIONS TO EMBRYOLOGY, No. 63.

THE DISTRIBUTION OF MITOCHONDRIA IN THE PLACENTA.

By G. B. Wislocki and J. A. Key.

Of the Anatomical Laboratory, Johns Hopkins University, and the Laboratory of Surgical Research, Harvard Medical School.

With one plate.

THE DISTRIBUTION OF MITOCHONDRIA IN THE PLACENTA.

Since mitochondria were discovered by Altmann in 1890, a voluminous literature has accumulated on this subject. Mitochondria have been described in almost every type of animal and plant cell and have been credited with functions varying from the transmission of hereditary characters to differentiation into neurofibrils, muscle fibrils, connective-tissue fibrils, and zymogen granules. Other observers believe that they are concerned in the metabolic processes of the cell. Recently the literature has been ably reviewed by Duesberg (1912) and Cowdry (1918), and therefore no attempt will be made to do so here.

The only reference to mitochondria in the placenta that we find in this extensive literature is a note by De Kervily (1916). This writer states that mitochondria are abundantly present in the Langhans cells of the human placenta throughout gestation. He makes no mention of their occurrence in the syncytium or other placental elements. It was consequently deemed of interest to investigate the mitochondria of the placenta more in detail and in different types of animals. We were guided in our choice by the classification of Grosser (1910), who arranged the placentæ of animals in an ascending scale dependent upon the union of the chorion with the uterine wall. Following this classification, we have chosen the pig, cat, guinea-pig, and human, as representing several rather distinct types.

According to Grosser, the pig represents the simplest type, in which the chorion is merely apposed to the folds of the uterine mucosa, in consequence of which the maternal and fetal blood-vessels remain widely separated. In the cat (Carnivora) the union is more intimate as the result of an invasion of the uterine mucosa and its stroma by the chorion, whereby the fetal ectoderm eventually surrounds the maternal blood-vessels, and the distance between the maternal and fetal circulation is greatly reduced. In the guinea-pig (Rodentia), a still more intimate fusion of the chorion with the uterine wall occurs. Here the chorion finally erodes the maternal vessels so that their endothelium disappears and the maternal blood flows through channels completely lined by fetal ectoderm. The barrier between the maternal and fetal blood-streams has been reduced to a single layer of chorionic epithelium and a delicate stroma. In the human, conditions are similar to those in the guinea-pig, although there are marked differences in the finer architecture.

MATERIAL AND METHODS.

A series of mature placentæ from the pig, cat, guinea-pig, and human were obtained and fixed fresh. Placentæ of earlier stages of development in the cat and the guinea-pig were also obtained in order to check up the various types of cells found in these animals.

Small pieces of the material were fixed either in the acetic-osmic-bichromate mixture, formalin-Zenker mixture of Bensley, or the various formalin-bichromate mixtures of Regaud. The sections were stained by the fuchsin-methyl green

method of Bensley, the copper-chrome-hematoxylin method of Bensley, the fuchsin-picric acid method of Altmann, the alizarin-anilin-gentian violet method of Benda, and the iron-hematoxylin method of Regaud. The best results were obtained by fixing according to method IV B of Regaud and staining by the fuchsin-methyl green method of Bensley. The great variation in the number and size of mitochondria renders differentiation by the iron hematoxylin method very difficult, as the minute mitochondria in some cells are completely decolorized while adjacent cells containing an abundance of large mitochondria remain deeply stained.

In Regaud's method IV B the fixative is made up of neutral formalin 1 part, 3 per cent aqueous solution of potassium bichromate 4 parts. The formalin is neutralized with magnesium carbonate. The tissues are fixed 4 days, the solution being changed daily; they are then mordanted for 8 days in a 3 per cent aqueous solution of potassium bichromate, washed 24 hours in running water, dehydrated in 50 per cent, 70 per cent, 95 per cent, and absolute alcohol, cleared in xylol and imbedded in paraffine.

Sections were cut 2 to 4 μ in thickness and fixed to the slide by the water-albumin method. The sections were freed from paraffine by xylol and passed through graded alcohols to water, placed for half a minute in a 1 per cent aqueous solution of potassium permanganate, then for half a minute in a 5 per cent aqueous solution of oxalic acid. They were then washed thoroughly in water and stained by Bensley's acid fuchsin-methyl green method, which consists of the use of two staining solutions: *i. e.*, (1) acid fuchsin, 20 gms.; anilin water, 100 c. c.; (2) 1 per cent aqueous solution of methyl green. In the use of this method the following procedure is to be carried out: Pour the acid fuchsin solution (a comparatively fresh solution must be used) on the slide and heat the slide over a flame until the solution begins to steam. Allow the stain to act about 6 to 8 minutes, heating 3 or 4 times to the steaming-point. Pour off the stain, blot, and then wash the slide thoroughly in distilled water. Blot and pour 1 per cent methyl green on the slide. This must be given time to stain the nuclei and protoplasm of the cells and is usually allowed to act about half a minute. Then blot and dehydrate in absolute alcohol, clear in xylol, and mount in balsam. If the sections are stained too deeply with methyl green the minute mitochondria are obscured. If such sections are dipped for an instant in 95 per cent alcohol part of the green is taken out and good differentiation can be obtained.

With this method the mitochondria are stained a bright red by the fuchsin and the protoplasm is light green or unstained. The chromatin of the nucleus stains green and the nucleoli either red or green. Unless otherwise stated, the following descriptions are based upon sections prepared by the above technique.

PLACENTA OF THE PIG.

In the pig the chorion is covered by a single layer of cuboidal or columnar cells which is applied directly to the uterine mucosa. A thin layer of structureless substance, the so-called *embryotrophe*, which stains light green, lies between the two tissues. The chorionic epithelium and uterine mucosa are thrown into inter-

locking folds. The cells of the chorion vary in height in different parts of each fold; at the apices of the chorionic folds they are of low columnar form, on the sides of the folds they are cuboidal, while in the depths of the folds a transition again occurs to columnar cells 20 to 30 μ in height. Situated in the basal half of each cell is a single large, oval nucleus which takes the green faintly, has a well-defined chromatin network, and one or two large fuchsinophilic nucleoli. The protoplasm of the basal half of the cell also stains a faint green. In the apical zone the protoplasm is clear and unstained. In many cells it is extensively vacuolated and degenerated cells with pycnotic nuclei and diffusely fuchsinophilic protoplasm are common. In a number of the chorionic epithelial cells a fuchsinophilic, colloid-like substance is found in the basal zone (usually between the nucleus and the base of the cell), varying in amount from one or two small granules to large masses 5 to 6 μ in diameter. The larger masses are surrounded by a clear zone as though they had contracted during fixation. The cells containing this colloid substance present no evidence of degeneration.

The mitochondria are concentrated in the apical half of the cells facing the uterine mucosa (fig. 1). They are quite abundant in the apical zone and appear as minute granules and very delicate, slightly curved rods. The rods and rows of granules are parallel to the long axis of the cell and appear as though streaming from its apex towards the nucleus. They steadily decrease in number as the distance from the apex increases, and it is rare to find any in the region of the nucleus and none is seen in the zone between the nucleus and the base of the cell. The cuboidal and low columnar cells of the sides and apices of the folds are similar to the high columnar cells in the depths of the folds, except that the various regions of the cell are less well marked and fewer cells contain colloid droplets.

The chorionic epithelium rests on a very thin basement membrane which is supported by a delicate connective-tissue layer containing the smaller fetal blood-vessels. This layer is composed of small spindle or stellate cells which do not differ from fibroblasts found in other tissues. They contain a few minute granular and rod-shaped mitochondria. The endothelium of the fetal vessels also contains a few granular and rod-like mitochondria and herein does not differ from ordinary vascular endothelium. This delicate connective-tissue layer merges into a thin layer of coarser fibrous tissue which contains the larger fetal blood-vessels, the cells of which are larger than those of the preceding one and contain larger and more abundant mitochondria. This fibrous layer is covered by the allantoic membrane, which is composed of a single layer of flattened epithelial cells resting on a delicate basement membrane. The allantoic epithelium is quite rich in minute granular mitochondria which are scattered uniformly through the protoplasm.

The uterine mucosa facing the chorionic epithelium is composed of a single layer of low cuboidal cells resting on a basement membrane. Each cell contains a single relatively large, round or oval nucleus which stains deep green and contains one or two large fuchsinophilic nucleoli. The protoplasm stains lightly with green and contains an abundance of rather coarse mitochondria (fig. 1). These are in the form of coarse granules and curved rods and are distributed quite unfiormly

through the protoplasm. Just beneath the basement membrane is a rich capillary network which rests on the connective-tissue stroma containing the maternal blood-vessels and uterine glands. The mitochondria of the connective-tissue cells and of the endothelium are similar to those of the corresponding cells in the chorion.

The uterine glands are lined by a single layer of medium-sized columnar epithelial cells 15 to 20 μ in height. Each cell contains a single round nucleus, located near the base of the cell, which stains light green and has a well-defined chromatin network and one to four fuchsinophilic nucleoli. The cell protoplasm stains a faint green and contains a rather large number of mitochondria, most of which are in the form of heavy, slightly curved rods (fig. 2). A few medium-sized granular mitochondria are found in either extremity of the cell. The rods are all arranged in the long axis of the cell and often a single rod extends the entire distance from the base to the apex of the cell. Many of these cells contain coarse globules of a substance similar in staining to the mitochondria. These are larger in diameter than the mitochondria and fairly uniform in size. In senile cells with pycnotic nuclei they may entirely replace the mitochondria. They are not uncommon, however, in the apical zones of apparently normal cells. In cells in a later stage of degeneration the globules are replaced by vacuoles.

The muscle fibers of the pregnant uterus contain numerous minute mitochondria in the form of short rods and granules. They are very numerous in a narrow zone around the nucleus and fairly numerous in the peripheral zone of the muscle fiber just beneath the fiber sheath. In lesser numbers they are scattered through the substance of the fibers.

PLACENTA OF THE CAT.

In the cat the placental labyrinth consists of alternating columns of fetal ectoderm and loose connective tissue. The maternal blood-vessels occupy the center of the ectodermal columns, while the fetal vessels are located in the connective tissue separating them. The layer of ectoderm surrounding the maternal blood-vessels is composed of giant cells and numerous smaller cells. The cytoplasm of the latter coalesces gradually, as gestation advances, to form a syncytium which incloses the maternal blood-vessels and the giant cells.

The cells lining the maternal vessels differ from ordinary endothelium in that their protoplasm is slightly more abundant and much richer in mitochondria (fig. 3). The mitochondria are so numerous that, unless the preparation is very thoroughly differentiated, the protoplasm of these cells stains a uniform deep red. In a well-differentiated section, forms varying from fine to coarse granules and curved rods are seen closely packed in the faint green protoplasm. The nuclei stain light green and have a green-staining nucleolus as in ordinary endothelium. In the earlier stages of the placenta these cells possess more abundant protoplasm rich in small mitochondria.

The giant cells, isolated or in small groups, alternate with the maternal vessels to form the center of the column. Occasionally, giant cells lie alongside of a maternal vessel between it and the border layer of fetal ectoderm. These are round to oval

in shape, vary from 15 to 40 μ in diameter, and contain from one to three nuclei. The average giant cell is about 20 μ in diameter and contains only one nucleus, which is large, round, and stains light green. Near its center is a single large fuchsinophilic nucleolus from which delicate strands of chromatin radiate to the nuclear membrane. The protoplasm stains faint green and may contain numerous minute vacuoles, especially in the older cells. The mitochondria are fairly abundant in the form of minute granules and rods arranged in an eccentric zone around the nucleus. The peripheral zone of the proptoplasm is entirely free from mitochondria or other granules. In some cells an eccentric ring of clear protoplasm is present between the nucleus and the zone of mitochondria. In cells containing two nuclei the zone of mitochondria may surround both and not invade the protoplasm lying between them; or, there may be a separate mitochondrial zone around each nucleus. In the giant cells of younger placentæ the mitochondria are slightly larger and more numerous in proportion to the amount of protoplasm and are not so definitely concentrated in the zone around the nucleus.

The small ectodermal cells form a layer which surrounds the giant cells and maternal vessels and separates them from the connective-tissue stroma containing the fetal vessels. At first these cells have quite distinct boundaries, but during the latter part of gestation their cytoplasm coalesces to form a syncytium which closely invests the giant cells and the maternal vessels. The smaller ectodermal cells are low columnar, about 10 μ in height, and are quite uniform in size. In the central portion of each cell is a single round nucleus which stains deep green and contains a single deep green nucleolus and several heavy masses of chromatin. The protoplasm contains a large number of vacuoles of various sizes, more abundant at the pole of the cells nearest the maternal vessels. In osmic preparations these vacuoles are seen to be filled with fat. The mitochondria, in the form of small granules and short rods, are fairly numerous and lie in the protoplasm between the fat vacuoles (fig. 3). They are not concentrated in any particular part of the cell. In the younger placentæ transition can be traced between the small ectodermal cells and the giant cells.

Lying between and over the columns of fetal ectoderm is a delicate connective-tissue stroma which contains the fetal blood-vessels. In the young placenta the cells of the stroma are quite rich in mitochondria, but in the mature placenta they possess no more mitochondria than do ordinary connective-tissue cells. The endothelial cells of the fetal vessels possess a few, but they are in no way remarkable.

The chorionic membrane of the cat consists of a single layer of cuboidal to columnar cells resting on a basement membrane supported by a very vascular connective-tissue stroma. On the opposite side this stroma is covered by the allantoic membrane. Numerous folds project from the chorionic surface toward the uterine mucosa.

The chorionic epithelium near the placental margin is composed of high columnar cells, but as the distance from the placenta is increased the cells become lower and more cuboidal. They are crowded together, their outlines being distorted by pressure from the surrounding cells. Many cells are apparently in process of

being pinched off by pressure from new cells arising next to the basement membrane. Usually, each cell contains a single nucleus which may be located in any part of the apex, depending upon the pressure from surrounding cells. However, cells containing two or three nuclei are not rare. The nucleus has a very indefinite limiting membrane and its outline is irregularly stellate, apparently from the pressure of vacuoles in the protoplasm which indent its surface and may almost completely divide it. It stains rather deep green and contains a single large fuchsinophilic nucleolus. The cytoplasm stains a faint green and is crowded with vacuoles of fairly uniform size, about 1 μ in diameter. In the apices of a few of the cells are large vacuoles containing fragments of phagocytosed red blood-cells. The mitochondria are not numerous and are seen as small granules and short rods lying between the vacuoles. They are not concentrated in any particular part of the cell.

The allantoic membrane consists of a single layer of flattened cells resting on a delicate basement membrane. The free surface of the cells is covered with debris. The nucleus is oval or flattened, stains a rather deep green and contains a single fuchsinophilic nucleolus and several irregular masses of chromatin. The pale-green protoplasm is finely vacuolated and contains a few small granular mitochondria.

PLACENTA OF THE GUINEA-PIG.

The vitelline membrane of the guinea-pig consists of a single layer of columnar cells of medium height resting on a basement membrane which is supported by a layer of connective tissue rich in small blood-vessels. The membrane is thrown into folds and numerous villi project from its surface. The columnar cells have dome-like apices which bulge out from the points of contact with the surrounding cells and this, in addition to the variation in height of the cells, gives an irregularly serrated edge to a cross-section of the membrane. . In the central portion of each cell there is a light-green nucleus which contains from one to four fuchsinophilic nucleoli. The protoplasm of the apical zone stains a light green, while that of the basal zone is clear and unstained. Two to six small, clear vacuoles are usually seen in the apical zone. In the more senile cells the protoplasm contains vacuoles of varying size. They may be quite large and may occupy any part of the cell.

A variable number of fuchsinophilic granules are constantly present in these cells. They vary from a few small granules to a large number of coarse globules 2 μ in diameter. When only a few are present they are located in the basal zone and around the nucleus, leaving the basophilic apex clear. They are apparently not products of degeneration, as they are present in all cells.

The mitochondria are not very numerous. They are mostly in the form of small rods, though a few minute granules are constantly present. In the average cell they are limited to the base of the cell and the zone around the nucleus and are distributed throughout the protoplasm. Apparently there is no quantitative relation between the mitochondria and the fuchsinophilic granules, as cells containing a large number of granules may contain either a very few or a relative abundance of mitochondria. Senile cells contain very few or no mitochondria. Senility, however, apparently does not affect the number of fuchsinophilic granules, as wide variations are found in cells with pycnotic nuclei and vacuolated protoplasm.

The vitelline membrane covering the placenta likewise presents a single layer of columnar cells. The cells here are considerably larger than those just described and in gross appearance resemble those of the chorionic membrane of the cat, in that they are crowded together with their nuclei at different levels and their cell-outlines are distorted by pressure from neighboring cells. Also, the older cells are constantly being constricted at their bases and pinched off by new cells arising next to the basement membrane.

Each cell possesses a single large oval nucleus which is usually found in the central zone of the cell unless influenced by pressure, when it may be found anywhere from the base to the apex. It stains deep green and contains one to three basophilic nucleoli.

The protoplasm stains faintly and in nearly all cells is extensively vacuolated, the vacuoles being largest and most numerous in the cells which are constricted at the base. Occasionally large vacuoles in the apical zone contain fragments of phagocytosed red blood-cells. The mitochondria, present as small granules and short rods, are fairly numerous, and especially so in the young cells next to the basement membrane. They are not concentrated in any particular part of the cells but are distributed uniformly through the protoplasm, the rods usually lying with their long axes parallel to the long axis of the cell. In the senile cells with irregular pycnotic nuclei the mitochondria are less numerous and many bulb-like swellings and mitochondrial vesicles are present. However, the mitochondria persist as long as the cell remains attached to the basement membrane.

Just beneath the thin basement membrane supporting these cells is a layer, one to four cells in thickness, of large round or oval giant cells. In the mature placenta most of these cells are senile and have vacuolated protoplasm and pycnotic nuclei, although a few can be found which appear to be quite healthy and normal. As a rule they are mononuclear, though cells containing two to four nuclei are occasionally seen. The nuclei are large, round to oval in shape, stain slightly with green, and contain a single large, deep-green nucleolus from which strands of chromatin radiate to the nuclear membrane. The protoplasm stains a faint green and is quite rich in minute granular and rod-like mitochondria. These may be concentrated in a zone around the nucleus or distributed uniformly through the protoplasm. In the degenerating cells few or no mitochondria can be seen. No other granules are visible in these cells.

Beneath the layer of giant cells is a well-defined layer of connective tissue which separates it from the cortical syncytium covering the labyrinth. From the inner surface of the cortical syncytium heavy bands of interlobular syncytium project into the labyrinth and divide it up into lobules. The structure of the cortical and interlobular syncytium is the same and they will be described together.

The syncytium is a coarse reticulum of heavy protoplasmic bands in which no cell boundaries can be distinguished. The interspaces of the reticulum are the sinusoids in which the maternal blood circulates and, as they are not lined by endothelium, the blood comes in direct contact with the syncytium. The small round to oval nuclei are very numerous and are distributed quite uniformly through the

protoplasm. They stain a deep green and contain a single large basophilic nucleolus. The protoplasm stains a bright green and is extremely rich in minute granular and short, rod-like mitochondria (fig. 4). These are quite uniform in size, the rods rarely being over 2 μ in length, and are distributed uniformly through the protoplasm. No other granules are demonstrable. In some areas the protoplasm is finely vacuolated and here the mitochondria are somewhat less numerous.

The labyrinth consists of a dense network of maternal blood-spaces, inclosed in a thinned-out syncytium, and fetal blood-vessels supported by a small amount of connective tissue. The syncytium resembles endothelium, but retains the richness in mitochondria (fig. 5) characteristic of the border and interlobular syncytium, and also exhibits a similar staining reaction.

The fetal blood is inclosed in delicate capillaries whose endothelium is similar to that found in other localities. Its nuclei and protoplasm stain a lighter green than do the nuclei and protoplasm of the syncytium and the protoplasm is about one-fourth as rich in mitochondria as is that of the syncytium. The mitochondria are much more variable in size and many curved rods, 6 to 8 μ long, are seen.

A section through the roof of the central excavation shows irregular columns of degenerating decidual tissue separated by papillary processes of fetal ectoderm, one or two cells in thickness. The fetal cells are small, cuboidal in shape, and contain relatively large round or oval nuclei which stain deep green and contain basophilic nucleoli. The small amount of green-staining protoplasm is quite rich in small granular and rod-like mitochondria. The cells of the decidua in this locality have highly vacuolated protoplasm and pycnotic nuclei. The mitochondria are limited to a few minute granules lying between the vacuoles in the protoplasm.

HUMAN PLACENTA.

The labyrinth of the mature human placenta is made up of chorionic villi surrounded by maternal blood-spaces. The fetal blood-vessels occupy the center of each villus and are supported by a rather coarse connective-tissue network. The villi are covered by a syncytium which everywhere separates the fetal vessels from the maternal blood. Just beneath this syncytium are varying numbers of clear Langhans cells. The syncytium is a layer of protoplasm of variable thickness in which no cell boundaries can be made out. The numerous nuclei are usually arranged in a single layer and are quite close together. However, in places where the syncytium is thick, as in the angle or at the junction of two large villi, there may be 4 or 5 layers of syncytial nuclei. They are small, round or oval in shape, stain deep green, and contain a medium-sized basophilic nucleolus.

The protoplasm of the syncytium takes the green faintly and is usually homogeneous, though in some areas it is finely vacuolated. It is very rich in minute granular and short rod-like mitochondria (fig. 6). These are distributed uniformly through the protoplasm and are so numerous and crowded that it is difficult to distinguish the individual granules except in very thin, well-differentiated sections. They are somewhat less numerous in the vacuolated areas.

The cells representing the remains of the Langhans layer lie just beneath the syncytium. They are usually seen in groups of 2 to 4 cells, though many isolated cells are present and occasional groups of 10 to 15 cells can be found. Many of these cells are senile and have vacuolated protoplasm and pycnotic nuclei. Others appear to be quite healthy and normal. They range from 10 to 20 μ in greatest diameter and vary in shape from isolated oval or spindle cells to rows of low cuboidal cells. The nucleus is rather large, stains light green, and contains 1 to 4 medium-sized basophilic nucleoli and a delicate chromatin network.

The protoplasm is clear and unstained and contains a moderate number of mitochondria. These are usually small, irregularly curved rods of moderate length, and many of them have small nodules at their extremities. A few granular forms are present. The mitochondria are sharply differentiated in the clear unstained protoplasm and this enables one to recognize these cells, regardless of their form or position. As the signs of degeneration increase the mitochondria decrease in number and size. No vesicles or other granules are seen in these cells. The Langhans cells are easily found in all mature human placentæ.

The decidual cells are found in an irregular layer next to the uterine wall as short columns projecting into the labyrinth. They are large round to oval cells 10 to 30 μ in diameter, and resemble somewhat the giant cells found in the placentæ of the pig and the cat, although they are entirely different in origin. They contain one or two large round or oval nuclei which stain a faint green and contain in turn one or two large basophilic nucleoli and a delicate chromatin network.

The protoplasm stains more intensely than the nucleus and is quite rich in minute mitochondria. They are in the form of short rods and granules and are distributed uniformly through the protoplasm. The protoplasm of many of these cells contains a colloid-like substance which stains deeply with fuchsin. It varies in amount from a few small droplets to large irregular masses 4 to 5 μ in diameter. An apparently normal cell may contain a large amount of this substance and the surrounding cells be entirely free from it. It is not present in the degenerating cells. Decidual cells are present in all stages of degeneration.

As senility advances, the nucleus becomes homogeneous and stains deep green, the protoplasm loses its affinity for the green, and the mitochondria decrease in number and size and finally disappear entirely.

CONCLUSION.

From the foregoing description, it will be seen that mitochondria are abundantly present in the various types of placentæ investigated. They are particularly numerous in those cells which form the barriers between the maternal and fetal circulation and through which the respiratory and nutrient interchange between the two organisms is constantly proceeding.

To review briefly, in the pig, mitochondria are very plentiful in the layers of epithelial cells, namely the chorionic ectoderm and the uterine mucosa which form the bridge between the maternal and fetal blood-streams. They are also found in great abundance in the epithelium of the uterine glands and this is significant,

because an important rôle is ascribed to these structures. The uterine glands of the pig's placenta are credited, according to several investigators (Ercolani, 1869; Bonnet, 1882) with liberating a profuse secretion, termed the uterine milk, which finds its way onto the surface of the uterine mucosa, where it is absorbed by the chorionic epithelium and thus constitutes one of the main sources of fetal nutriment.

Mitochondria are equally numerous in the placenta of the cat. They occur chiefly in the broad layer of chorionic epithelium which intervenes between the circulation of the mother and that of the offspring. They are also abundant in the delicate layer of endothelium which incloses the maternal blood-channels. Little has been reported concerning the histochemistry of the cat's placenta beyond the fact that the chorionic epithelium contains numerous fat droplets (Melissenos, 1906). Recently, one of the authors (Wislocki, 1920) has studied the behavior of the cat's placenta toward trypan blue injected into the maternal blood-stream. It was found that trypan blue does not pass from mother to fetus but is stored in the form of minute granules in the cytoplasm of the chorionic epithelium and the endothelial cells which line the maternal blood-vessels. These are the cells whose cytoplasm, as described, is filled with mitochondria.

In the guinea-pig mitochondria are found in great profusion in the syncytium which comprises the bulk of the placental labyrinth and separates the maternal from the fetal blood-stream. Fat and glycogen are also readily demonstrable in the syncytium and trypan blue is deposited there when injected into the maternal circulation. The placenta of the guinea-pig possesses numerous degenerating cells of both fetal and maternal origin and it is interesting to note that these cells lose their mitochondria while undergoing degeneration.

In the human placenta, also, it is the syncytium and Langhans cells which contain mitochondria in greatest abundance. It need only be emphasized that these are the cells which separate the two circulations and in which products of metabolism, such as glycogen (Driessen, 1907) and fat (Hofbauer, 1910) are amply demonstrable.

In conclusion it is interesting to consider what significance can be ascribed to the mitochondria in the placenta. It is striking that they are most numerous in those cells of the placenta which are presumed to play an important rôle in the metabolic transfer between the two organisms. From observations such as we have made, however, it is impossible to assign to them any specific function. The only conclusion one can safely draw concerning mitochondria is that they represent material which is probably used in the multiphasic activities of the placental cells.

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EXPLANATIONS OF FIGURES.

FIG. 1. Section of the placenta of the pig showing the apposition of the chorion to the uterine mucosa. The chorionic epithelium and uterine mucosa contain numerous mitochondria.

FIG. 2. Section of a uterine gland from the pig's placenta showing mitochondria.

FIG. 3. Section of the placenta from a cat, nearly half term, showing the mitochondria in the endothelium of the maternal blood-vessels and in the chorionic epithelium.

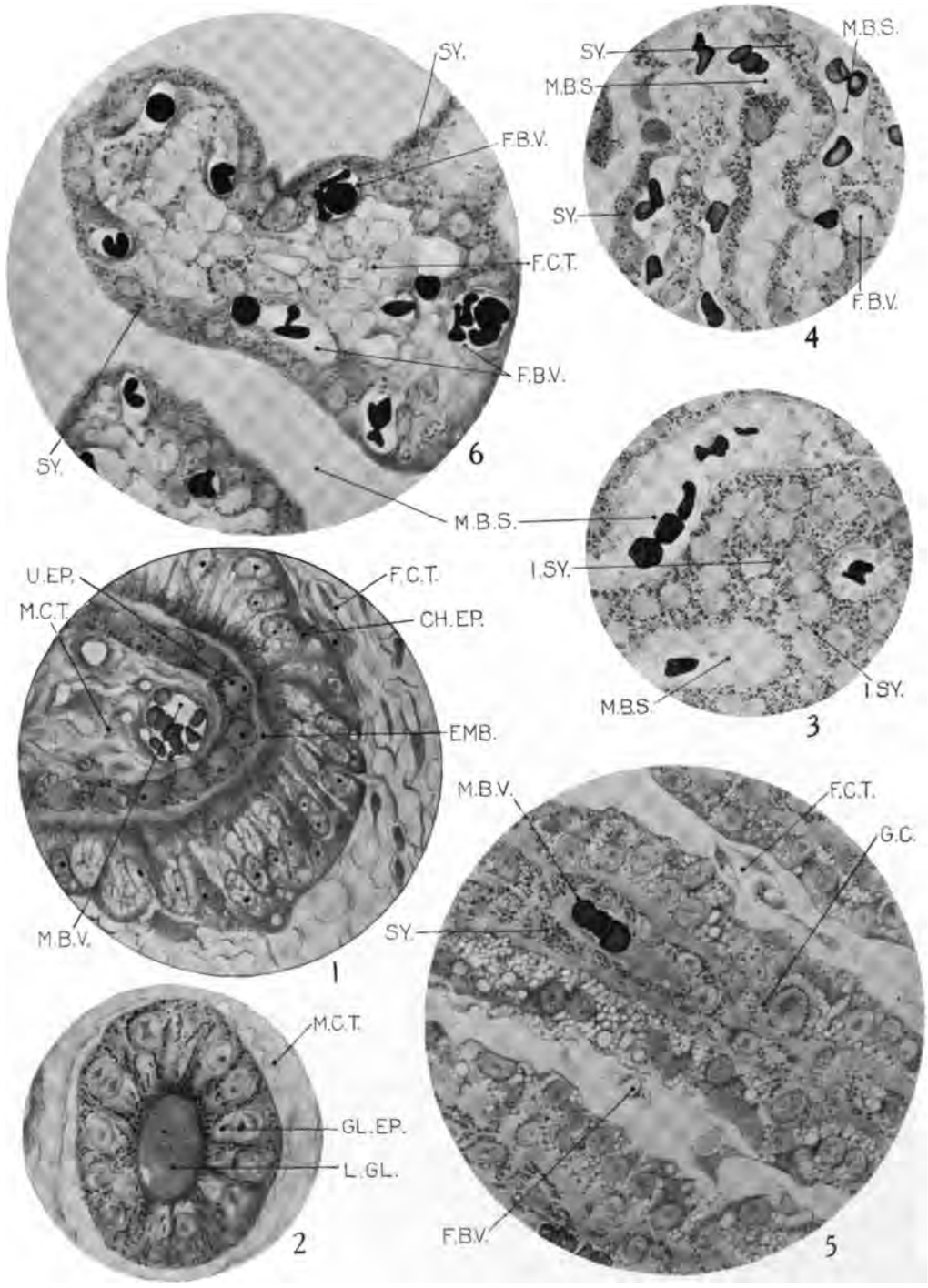
FIG. 4. Section of the placenta of the guinea-pig, showing abundance of mitochondria in the interlobular syncytium.

FIG. 5. Section of the placenta of the guinea-pig, showing mitochondria in the syncytium of the labyrinth.

FIG. 6. Section of villus from human placenta showing mitochondria in the syncytium.

ABBREVIATIONS.

CH. EP.,	Chorionic epithelium.
EMB.,	Embryotrophe.
F. B. V.,	Fetal blood-vessel.
F. C. T.,	Fetal connective tissue.
G. C.,	Giant cell.
GL. EP.,	Glandular epithelium.
I. SY.	Interlobular syncytium.
L. GL.,	Lumen of gland.
M. B. S.,	Maternal blood-space.
M. B. V.	Maternal blood-vessel.
M. C. T.,	Maternal connective tissue.
SY.,	Syncytium.
U. EP.,	Uterine epithelium.



CONTRIBUTIONS TO EMBRYOLOGY, No. 64.

CYCLIC CHANGES IN THE OVARIES AND UTERUS OF THE SOW, AND THEIR RELATION TO THE MECHANISM OF IMPLANTATION.

By George W. Corner,

From the Anatomical Laboratory of the Johns Hopkins Medical School.

With four plates and two figures.

CYCLIC CHANGES IN THE OVARIES AND UTERUS OF THE SOW, AND THEIR RELATION TO THE MECHANISM OF IMPLANTATION.

1. GENERAL.

INTRODUCTION.

The undertaking set forth in these pages is simple in plan, namely, to follow in one mammal (the sow) the anatomical changes in uterus and ovary which underlie the reproductive cycle. The prospective value of such a study may deserve a word of explanation; it arises from the fact that in different organisms there are varying forms of expression of the cyclic sexual processes. Even within the order of mammals, the reproductive cycle of the female is obscured by as yet unresolved dissimilarities and no satisfactory correlation has been made between phenomena so unlike as the urgent rutting of deer, the inconspicuous œstrus of rodents, and the menstrual cycle of the catarrhine apes and man. In our own species we have only conflicting conjectures as to the time of ovulation and the meaning of menstruation, although exact knowledge of this subject would be of value not only for its own sake, but also for the calculations of the embryologist and the gynecologist. The need, therefore, is for a detailed study of individual species, including the determination of the œstrous period and the time relations of ovulation, growth, and retrogression of the corpus luteum, tissue changes in the uterus, and the progress of the ova, whether fertilized or unfertilized.

Some such account has been pieced together for five species, though not with equal completeness: a marsupial, *Dasyurus viverrinus* (Hill and O'Donoghue, 1914); the rabbit (Niskoubina, 1909; Ancel and Bouin, 1910; etc.); the guinea-pig (Loeb, 1911, 1914; Stockard and Papanicolaou, 1917; Ishii, 1920); the rat (Long and Evans, 1920–1921)[1]; and the dog (Marshall and Jolly, 1906; Marshall and Halnan, 1917; Keller, 1909). Something is known of the cycle of ovulation but not of the uterine changes in several other mammals, while in man we know much about the histological cycle of the uterus and almost nothing of the ovarian sequence.

Choice of the domestic pig as subject for a similar study was determined by a combination of circumstances which seemed to outweigh the disadvantages of large size and commercial restrictions upon the collection of material. In this animal œstrus is regularly periodic, frequent, and outspoken; ovulation is spontaneous; study of the ova and embryos is facilitated by the large litters; the ovaries of the ungulates are notably uncomplicated, as contrasted, for instance, with those of the rodents; and the uterine mucosa is of the non-deciduate type,

[1] I am indebted to Professors Long and Evans for the opportunity to study in manuscript their forthcoming definitive account of the œstrous cycle of the rat, based on their preliminary studies referred to in the appended bibliography.

with a simple, diffuse placenta. In short, the problem is here reduced to its lowest terms, and the solution promises, therefore, to be the more useful as a contribution to the general theory of the reproductive cycle.

EXTERNAL MANIFESTATIONS OF THE REPRODUCTIVE CYCLE IN SWINE.

Investigations in the physiological anatomy of the reproductive system must depend upon material collected at accurately determined periods of the ovarian-uterine cycle. For this reason it will be necessary to review briefly the well-known facts of the sexual manifestations of swine.

Sexual maturity is attained before the age of one year, sometimes as early as 4 months, often before the uterus has attained its full adult dimensions. Maturity is characterized by the recurrence of periods of sexual activity ("heat," or œstrus) at intervals of 2 to 4 weeks, the usual interval being 21 days. An exact study of the duration of the œstrous cycle has been made by Struve (1911), whose results agree closely with the figures just given; his computations give a mean interval of 20.66 ± 0.205 days, with a standard deviation of 2.36. The curve of frequency distribution of observed cases shows the shortest interval to be 15 days, the longest 30 days, but 75 per cent of the animals fall within the limits of 18 to 23 days. The duration of œstrus is commonly 3 days, during which time the rutting sow exhibits excitement in the presence of the male, with ready acceptance of coitus. If no male is present the sow will follow the other females about the pen, sniffing at their genitals and frequently going through an imitation of the sexual act by "riding" upon the others; or she may at times be the recipient rather than the donor of these attentions. In a large herd without boars the females in heat will often be found in a separate group apart from the others, where for hours at a time the exhibition of the œstral urge continues, interrupted only by siesta or feeding time, until at the end of 3 days, or occasionally longer, it subsides, to be followed by an interval during which sexual activities are in abeyance.

At the height of œstrus the vulva is often slightly everted, swollen, and reddened, and there is sometimes a slight serous or mucous vaginal discharge. Occasionally the discharged fluid is flecked or stained with blood, but from internal examination of the reproductive tract it is apparent that in these cases the bleeding is of external origin, caused no doubt by trauma to the vulva; in this detail, as in all others of the description just given, the external manifestations of the sexual cycle are in complete contrast to those displayed by the human and other primate races.

The 21-day cycle appears to continue regularly throughout the year unless interrupted by pregnancy. In pregnant animals received at the packing-houses, fetuses are found in all stages of development without much regard to the time of year. Pregnancy can begin only at an œstral period, since then alone is copulation permitted. The span of gestation is 16 to 17 weeks, usually 116 to 120 days. As with other mammals, the œstral periods do not occur during pregnancy, but according to Struve (1911), œstrus recurs about 4 to 9 days after parturition.

The number of young in a litter varies from 1 to 23, the mean being from about 7 to 9 in different American breeds, according to Surface (1909), who has made a biometric analysis of the data upon this subject.

COLLECTION OF THE MATERIAL.

The first step in correlating the organic changes with the outwardly visible functional events which have been recounted is, necessarily, to determine at what point of the cycle ovulation takes place. The results of this part of the investigation, and of a study of the origin of the corpus luteum and its alterations during pregnancy, have already been described in previous publications by the writer (1915, 1917*a*, 1917*b*, 1919) and need only be summarized here.

It was found, as had been expected by analogy with previously known species, that the period of œstrus is the time of ovulation. In the stockyards the condition of heat was observed in about 30 animals, which were suitably marked and then were followed through the processes of the abattoir until the genitalia were obtained from the butcher's hands. In such of these animals as were killed during the three days following the onset of œstrus the ovaries contained either mature or recently collapsed Graafian follicles. This much had in fact already been proved by Lewis (1911) in a publication at the time unknown to the writer, but we were able to strengthen the evidence by actual recovery of the ova from the Fallopian tubes. By the use of simple technical methods, which will be described later, a series of segmentation stages and also of unfertilized ova was obtained for study and correlation with the corpora lutea. By good fortune the packing-house where these first steps were taken was not at the time under great stress of production; a few of the swine were retained in the corrals as long as 11 days after the onset of œstrus, and it was therefore possible to follow the development of the corpus luteum up to about the tenth day. It was not until the adoption of a rapid method of locating ova and blastodermic vesicles in the large uterine chambers of the sow that the fate of the ova after their exit from the Fallopian tubes could be continuously followed; but the writer already had in hand the ovaries of sows in all stages of pregnancy from the third week until parturition, to the number of about 140. Upon the sum of this material two previous contributions were based (1915, 1919), covering, therefore, the corpus luteum of pregnancy from the day of ovulation until after parturition and the corpus luteum of unfertilized ovulation until the tenth day of its development.

It was, of course, impossible at the stockyards to follow animals into the latter half of the cycle. Such material, however, was made available by the generous interest of Mr. Walter N. Cooper, manager of the American Feeding Company, of Baltimore, who extended every facility for the undertaking. At the establishment of the company, about 20 miles from Baltimore, large numbers of pigs were kept from an early age until they attained a profitable weight by the consumption of table refuse and other edible garbage collected in the city. The writer made a series of trips to the piggery farm on alternate days throughout a period of 3 weeks,

and at each visit selected, with the aid of Mr. Cooper and his assistants, 3 sows which were evidently in active heat. These were marked by ear-tags and isolated from the herds in a special pen. The date of cessation of œstrus was noted by report of the piggery laborers and by personal observation on the days of visit.

At the end of 3 weeks 22 animals had been thus isolated, forming a series of all stages of the cycle. These were then purchased by the University and resold to a packer near the laboratory, by whom all of them were killed on the same day, and the internal genitalia were thus recovered for study. The sows were all in good condition, readily passing government inspection. They were of various breeds and crosses, but were nearly uniform in age (about 10 to 12 months) averaging over 180 pounds in weight, indicative of fairly mature state.

These 22 animals have served as a basis for all statements in the following pages as to the time relations of the reproductive cycle, but, owing to the variability of the intercœstral period, it chanced that none of them was killed within the first 3 or 4 days following ovulation. This gap in the series of uteri might have been filled by specimens from the first collection of animals mentioned above, but unfortunately all the uterine preparations of those sows had been put permanently beyond the reach of study by the departure for the war of a pupil to whom they had been intrusted. For this reason a further series of 30 specimens was collected from the slaughter-house. These were not obtained from animals that had been observed during life, but were at least approximately dated by the fact that early corpora lutea were present in all and that the ova or early embryos were recovered from each specimen. By the microscopic structure of the corpora, as worked out from the previous specimens, and by the condition of the ova, it was possible to rank them in a series and to discover from them the earlier uterine changes following ovulation.

It is obvious that there is a limit to the chronological accuracy of data obtained in the various ways described above. Students of the human ovary and uterus may perhaps think enviously of the opportunity afforded in the packing-house and stockyard to collect fresh organs, datable more or less closely, from young, healthy, mature animals to the number of twelve score and to see similar but undated specimens totaling almost 12,000; on the other hand, no such exactness is possible with the large animals of commerce as with animals like the rat, where the method of Stockard, as applied by Long and Evans, permits prediction of the time of ovulation within one hour. In this series of swine it has not been possible, in most cases, to observe œstrus from start to finish, nor do we yet know more than approximately the relation of the moment of ovulation to the usual 3 days of œstrus. Moreover, we are dealing with variable quantities in the duration of the intercœstral period and the rate of development of the fertilized ova, both of which factors have been used in establishing correlations. However, the reader will probably not be led astray if we estimate that all references to a given day, assuming a 21-day cycle as a working basis, imply a possible error of ± 1 day, perhaps even of ± 2 days as we approach the latter days of the intercœstral period.

METHODS OF PREPARATION.

Since recovery of the ova and early embryos by means of serial section of the tubes and uteri is out of the question in so large an animal as the sow, a procedure was devised which grew out of a suggestion by Professor Evans based upon the practice of Martin Barry (1839), one of the earlier workers on the problems of ovulation, who obtained the tubal ova of rabbits by stroking the tubes with a rod in order to express their contents into a dish. Our improvement, which we have since found had already been used by Sobotta (1897) and others, consists simply in washing out the Fallopian tubes with a stream of isotonic salt solution.

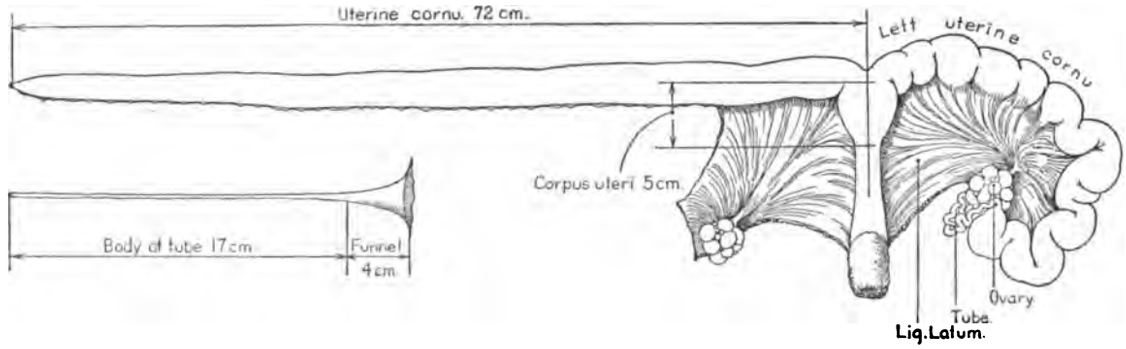


FIG. 1.—Diagram showing form and dimensions of the uterus and Fallopian tubes of the sow, drawn from an average specimen taken from a young mature animal.

Reference to figure 1 will make the following description clear. The Fallopian tube is freed from the mesosalpinx by cutting the latter off close to the tubal border and is detached from the uterus at the junction of the tube and cornu. The now straightened tube is suspended by one hand over a Syracuse dish and is filled, by means of a pipette, with sufficient normal saline solution to distend the lumen moderately. The narrow uterine end of the tube prevents the fluid from draining into the dish until the tube is gently "milked" out with the fingers. If ova are present they usually pass out with the first drops of fluid, but in some cases four or five washings are necessary to recover all that are present in the tube. The ova are located in the dish by inspection of the washings under a low-power microscope.

This method is rather awkward when applied to the whole uterus because of the large quantity of fluid required to distend the canal, amounting often to 300 c.c. For this reason recourse has been had to a plan by which a small amount of fluid (10 to 30 c.c.) is caused to distend successive short portions of the uterus. The uterine cornu having been uncoiled by cutting it from the mesometrium, a stout clamp is applied to it a few centimeters below the ovarian extremity and the distal portion is dilated with the salt solution; another clamp is now applied a few centimeters below the first, which is removed, and the fluid is forced by gravity and by digital pressure into the second section of the uterus, which is thus in turn distended, and so on. In this manner any ova or unattached embryos which are present are picked up and carried along until the fluid has passed through the whole length of the uterus, and are then deposited with the washings in one or two Syracuse dishes.

The washing-out method is very satisfactory for recovering ova and vesicular embryos from the uterus, but if it is inadvertently applied to a specimen containing embryos of the latter part of the second week or of the third week, which have undergone the extraordinary extension in length of the chorion which is characteristic of the pig and some other ungulate species, the result will be an almost hopeless tangle of chorionic membranes. Therefore, it is a wise precaution, when expecting early embryos of uncertain age, to slit up the cervical end of the uterus for a distance of several centimeters, watching for the delicate, glutinous, threadlike appearance which marks the early chorion. If none is found, then any still earlier embryos which may be present are presumably grouped somewhere in the remaining part of the uterus and may be recovered by washing.

Embryos of the third week and older have been removed by the usual method employed by embryologists, namely, by cautious opening of the uterus under salt solution in a well-illuminated glass dish against a dark background. The salt solution used in the work has generally been prepared according to the formula of Locke, except for the omission of glucose.

The large size of the mature Graafian follicle also presents difficulty when it is desired to locate the undischarged ovum. The use of serial sections of the whole follicle is sometimes necessary, in which case much labor can often be saved by embedding in celloidin and employing an assistant to cut the sections, which the observer examines in turn as they are cut, until the cumulus oöphorus begins to appear in the sections, after which still greater care is used until the ovum itself is reached. Another method is to lay the hardened ovary into serial slices of about 1 mm. thickness and then to search the follicle walls in the slices under the binocular microscope until the cumuli are found. The ovum-bearing portion of the follicle is cut out of the slice and sectioned in paraffin. As there are usually several follicles in each ovary, the writer's practice has been to try the latter method, reserving enough of the follicles to assure success by the former plan if the latter fails.

Histological preparations have been made by the usual methods. Bouin's picro-acetic-formol has been the chief fixing fluid, supplemented, of course, by a variety of others for special purposes. The stains used will be specified in connection with the illustrations. When it was necessary to wash out the uterus, a block for histological preservation was first cut from the middle of one cornu in order to spare the tissues any harm resulting from the fluid and from the pressure; the portions remaining were then separately washed out for the ova.

As it is difficult to cut thin sections of the sow's uterus except from very small blocks, complete transverse sections of each uterus were made by the celloidin technique to serve as reference for the small blocks used for study of cytological and microchemical details.

The writer's thanks are due to the American Feeding Company and to Hohman & Sons of Baltimore, to the Western Meat Company of San Francisco, and to Joseph Stern & Company of New York City for opportunity to collect this material; and to the Department of Embryology of the Carnegie Institution for technical assistance.

GROWTH OF THE GRAAFIAN FOLLICLE AND MATURATION OF THE OVUM.

The ovaries of the large animals are not well suited to the study of the various problems connected with the growth of the Graafian follicle, on account of the obvious physical difficulties which prevent systematic examination of large numbers of follicles and ova. Sufficient information has been gained, however, with respect to the pig, to supply an outline of follicular growth and to correlate its events roughly with those of the œstrous cycle. It appears that normal follicles of maximum size are found only during the period of a few days previous to ovulation. At all other times there is what might be termed a reserve stock of smaller follicles in the ovary, forming a series of all sizes from the microscopic primordial stage to a diameter of about 5 mm. Judging from the evidence of the 22 mature sows mentioned above, it seems that practically all actively functioning ovaries contain a number of follicles of 3.5 to 5 mm. diameter, which are in readiness for the final enlargement which they are destined to undergo just before rupture. Follicles of larger size are usually found to be atretic, although in a few cases there are normal follicles of 6 and even 7 mm. diameter during the interœstral period.

At a time not as yet accurately determined, but which can not be more than 2 or 3 days before the onset of œstrus, there begins a rapid enlargement of those follicles which are to discharge their ova, bringing them to a diameter of 7, 8, or even 10 mm.; and at the same time there is a series of histological changes, which, in the smaller animals, have long been known to be connected with the maturation of the ovum. These consist of growth of the theca interna by enlargement of its cellular elements and of a partial dissolution of the cumulus oöphorus by separation of its cells, so that the ovum is finally almost freed from its originally firm anchorage to the follicular wall. A full description of these changes will be found in the author's paper of 1919. The ovum itself goes through the first stages of the process of maturation, its nucleus moving toward the periphery of the cell, to form first the typical germinative vesicle, and then to undergo mitosis and extrusion of the first polar body.

The study of new material has not modified the opinion already expressed in a brief note (1917) that the process of maturation of the ovum follows in the pig the same course as in other mammals. At the moment of rupture of the follicle the first polar body has been discharged and the second polar spindle has been formed, but completion of the second polar body does not take place unless the ovum is fertilized.

Rupture of all the follicles seems to take place simultaneously or at least within a brief space of time, for in a careful examination of perhaps 200 pairs of ovaries containing recently ruptured follicles there has been only one case in which there were also normal, mature, uncollapsed follicles. A few other specimens which appeared to be of this sort proved upon section to be atretic. This conclusion is contrary to that of Küpfer (1920), who thinks that an appreciable space of time may be required for the rupture of a group of follicles, but the specimens upon which he bases this statement have apparently not been subjected to microscopic exami-

nation. Corner and Amsbaugh (1917) concluded, from a study of 10 animals, that ovulation probably occurred on the first or second day of œstrus; but the exact time of onset of heat in these animals could not be stated with great exactness, since the pens were visited but twice daily, and in some cases less often. A somewhat different conclusion is drawn by Lewis (1911) from the results of his experiments, unfortunately not known to us at the time of our work. His material consisted of 23 sows, in which the onset of heat and the time of copulation were noted with exactness, the animals being killed at varying times thereafter. In those killed before 30 hours after onset of œstrus the follicles were not ruptured (with one exception), but in most of those killed between 30 and 48 hours the follicles had collapsed. In one case ovulation had not occurred at 70 hours, but as no microscopic examination is mentioned, we can not exclude the possibility that this last case is one in which the follicles had undergone atresia instead of rupture. Lewis's interpretation is that "the ovum (in hogs) is not liberated from the ovary until the last part of the period of heat," but from his table one is forced, rather, to conclude that ovulation usually occurs during the second day of the period of œstrus.

FATE OF THE OVUM: PASSAGE AND ATTACHMENT OF THE EMBRYOS.

The time necessary for the passage of the ovum through the Fallopian tube has been set by Assheton (1898) at 3 days, and the present writer's findings are in accord with this statement. This journey of 18 or 20 cm. is therefore traversed in the same time as the far shorter distance in a small mammal like the mouse. If copulation has occurred, the conjugation of ova and spermatozoa takes place in the Fallopian tube and segmentation reaches the stage of 2, 4, or 6 blastomeres by the time the uterine end of the tube is reached. About the fourth day the ova enter the uterus.

Much interest attaches to the fate of the ova when copulation does not occur. Degenerating ova are but rarely found in the tubes, and one is therefore forced to suppose that all the ova pass into the uterus, whether or not the sow has been impregnated. This conclusion is strengthened by the fact that if from the run of sows at the abattoir one selects those whose ovaries contain fresh-looking solid or nearly solid corpora lutea (*i. e.*, 5 to 7 or more days after ovulation), a considerable proportion of these will be found, upon careful search, to contain either normal-looking or degenerating unfertilized ova in the uterus. Scores of such observations, confirmed in numerous cases by microscopic estimation of the age of the corresponding corpora lutea, make it seem clear that the unfertilized ova regularly pass into the uterus and degenerate there, not disappearing completely, however, before at least the seventh day after ovulation.

Some information as to the probable time of their final dissolution can be obtained from the following table of cases in which the ova were sought in the uteri of animals whose last œstrous period had been observed, and which had not been impregnated.

Degeneration of ova in non-pregnant animals.

Serial No.	Calculated days after onset of œstrus.	Age of corpus luteum estimated from structure.	State of uterine ova.
1	3 to 5	4 to 6	Present, not degenerated.
2	3 5	6 7	Not found.
3	6 8	7	Present, not degenerated.
4	6 8	7	Present, not degenerated.
5	8 10	7 about.	Not found.
6	8 10	7 about.	Not found.
7	6 8	7	Present, degenerating.
8	6 8	7 or more.	Not found.
9	8 10	7 or more.	Present, degenerating.

No ova were found in animals of later date.

We shall not be far wrong, therefore, in assuming that the unfertilized ovum of the sow disappears by degeneration *in utero* at about the seventh or eighth day after its discharge from the follicle. Degeneration of the unfertilized ovum is characterized by division of the cytoplasm into rounded masses, which are usually not uniform in size, but sometimes are sufficiently regular to simulate segmentation. These masses ultimately contract so as to present a shrunken appearance within a zona pellucida which is often swollen but at the same time less refractive. The zona pellucida frequently loses its spherical shape and at the last may present only a vague, flattened halo, containing minute punctate granulations, around a few shrunken masses of cytoplasm.

Of still more importance, in affording a basis for correlating the anatomical events of the reproductive cycle, is the question of the rate of growth and the time of implantation of the fertilized ovum. Fortunately for our purposes, pig embryos of known ages have now been seen at all stages of early development. The present writer, with Amsbaugh (1917), had the opportunity of completing the series by the observation of ova immediately after fertilization, before conjugation of the pronuclei. The careful studies of Assheton (1898) on about 100 embryos carry the history from the stage of two blastomeres until the tenth day, and 30 more specimens of the ninth, tenth, and eleventh days have been described by Weysse (1894). From the fourteenth to about the twenty-fifth day we have the detailed data of Keibel's Normentafeln (1897). The following account of the early embryology of the pig is based upon the contributions just mentioned and upon personal study of a series of specimens of all stages.

Segmentation begins in the tube and continues after passage of the ova into the uterus; no specimen beyond the stage of 6 blastomeres has yet been observed in the tube. Assheton found that various sows killed on the sixth day contained uterine embryos from 6 blastomeres to fairly well-developed blastodermic vesicles. By the seventh day the zona pellucida has disappeared and two layers, epiblast and hypoblast, may be distinguished in the inner cell-mass of the vesicles. By the eighth or ninth day the vesicles begin to be wrinkled and then to undergo the remarkable elongation which is so characteristic of early pig embryos. By the

twelfth day the vesicles are 10 to 12 mm. in length by about 3 mm. in diameter at their widest part; and by the fourteenth day they are 20 to 30 cm. long, with embryonic areas showing the neural groove well developed and possessing 1 to 5 somites. At 17 or 18 days the uterine cavity is completely filled by the embryonic envelopes, which, when their accordion-like folds are eliminated by tension, are found to have reached their full length of 30 to 40 cm.

It is important to remember that the relations between maternal and fetal tissues are of the simplest character in this species. No decidua is formed from the uterine mucosa, which retains during gestation a structure not widely different from that of the non-pregnant period, and the chorion simply becomes applied, over practically its whole surface, to the epithelium of the uterus (fig. 33, pl. 4). Moreover, the chorion itself remains a simple one-layered membrane, against the inner surface of which, after the third week, the vascular allantois is intimately applied. Nutritive substances passing from mother to embryo must then traverse both the uterine epithelium and the columnar chorionic epithelium in order to enter the fetal (allantoic) vessels. At all periods of pregnancy the chorion is readily detached by gentle traction and at parturition the fetal membranes separate from the uterine wall without lesion or hemorrhage, leaving an intact surface—an arrangement which seems excellently adapted to minimize the strain of giving birth to litters sometimes as numerous as 20 or more.

In view of the diffuse non-deciduate placentation, it is not possible to define sharply the time of implantation. We can say only that after about the tenth day growth of the vesicle is so extensive that it must then be considered dependent upon maternal nutrition rather than upon its own resources; by the thirteenth day, if not earlier, its position is fixed; by the fifteenth the chorion has come into relation with a large part of the uterine mucosa; while during the third week the allantois begins to line the inner surface of the chorion and hence brings fetal and maternal tissues into their definite relationship. It is the period from the tenth to the fifteenth day that is most nearly comparable to the much more precisely limitable act of implantation which occurs in animals like the rabbit and guinea-pig, and presumably in man.

II. OVARY.

ORIGIN AND COMPLETED STRUCTURE OF THE CORPUS LUTEUM.

The author's views as to the origin of the corpus luteum have been fully confirmed by the examination of new material obtained in connection with the study of the uterine cycle, amounting to about 35 animals taken in the first 8 days after ovulation. For a detailed description of the changes undergone by the discharged follicle during its conversion into the corpus luteum, the reader is referred to the author's article of 1919; at the present time a brief summary will suffice as introduction to an account of the subsequent history and degeneration of the corpus luteum.

When the Graafian follicles collapse at the time of rupture, the extrusion of the follicular fluid and the contraction of smooth-muscle cells in their walls reduce their diameter of 8.5 to 10 mm. to 4 to 6 mm. By the end of one week's growth, however, the corpora lutea are again 8 to 9 mm. in diameter, and if pregnancy ensues there is a further growth until, after 2 or 3 weeks, the maximum size of 10 to 11 mm. is reached. The membrana granulosa is retained intact, except for loss of the cumulus oöphorus, after rupture of the follicle. Its cells increase in size without division, their cytoplasm becomes laden with lipoid substances, and they become the larger elements, commonly known as "lutein cells," in the fully formed corpus luteum. During this process the membrana granulosa is invaded by blood-capillaries from the theca interna, which ramify to form an extensive vascular plexus throughout the new structures. The large lipoid-laden cells of the theca interna of the Graafian follicle are increased in number by mitotic divisions and pass into the corpus luteum to become lodged between the granulosa cells throughout the whole structure. There is no evidence that the cells of the theca interna are ever converted into fibroblasts of the usual spindle-cell type or that they lay down the fibrils of the close-meshed reticulum which is present in the corpus luteum.

The time relations of these changes may be given as follows: ovulation probably occurs on the second day of œstrus; invasion of the granulosa by blood-vessels and theca interna cells begins on the third or fourth day after onset of œstrus and is completed about the sixth or seventh day; by the seventh day the corpus luteum is usually solid and its cells have become fully differentiated.

Küpfer (1920) has given a valuable series of colored plates representing the gross appearance of the follicles and of the corpora lutea at various stages of development and retrogression. Unfortunately, the figures were not made from specimens of known relation to the œstrous cycle, and for this reason we have provided (plate 2) drawings of ovaries of 3 sows killed at known periods: during œstrus (fig. 9); at 8 days after ovulation, with completely solidified corpora lutea (fig. 10); about 17 days after ovulation, with degenerating corpora lutea and a new group of follicles beginning their precestrous enlargement (fig. 11).

The corpora lutea of a sow at the tenth day after ovulation are very conspicuous objects by reason of their size, reaching 8 to 9 mm. in diameter and projecting nearly all their bulk from the ovary, so that when numerous corpora are

present the remainder of the ovarian substance is dwarfed in comparison. At this stage the corpora are nearly always solid, though at times one or two in an ovary may remain slightly cystic. The cut surface bulges from the capsule and presents a somewhat velvety texture of pink color without trace of the yellow and orange pigments which are so characteristic of the corpus luteum in the bovine and human ovaries. The consistence of the corpus luteum at this time is not unlike that of the pig's liver.

In microscopic sections (fig. 3, pl. 1; fig. 12, pl. 2) the granulosa lutein cells form the most conspicuous element of the tissue, since they are distinguished both by their large size (reaching the diameter of 30 to 40 micra) and by the elaborate cytoplasmic patterns which they contain after fixation in slow aqueous fixatives like formol, Bouin's picro-formol-acetic fluid, etc. As previously described (1919), the granulosa lutein cells of certain species contain a large amount of a lipoid or mixture of lipoids, probably associated with proteins, which is sufficiently oily to round up into droplets when the tissue is submitted to the action of water. The droplets thus produced usually surround the pre-existing globules of neutral fat, which are also present in considerable numbers in the granulosa lutein cells of swine, but these latter are readily removed by the alcohol, ether, and xylol of the usual histological procedures, and hence the final appearance is usually such as shown in figure 12 (*gr. l. c.*), in which the lipoid droplets are seen as hollow spheres (appearing as rings in thin sections) more or less retracted from the surrounding cytoplasm. The bodies in question are not seen in fresh tissues, nor in cells fixed very promptly in rapid coagulants like osmium tetroxide, which presumably precipitate the proteids before the oil droplets round up. The appearances described, therefore, are simply the result of methods of fixation which do not preserve certain obscure lipoids in their natural diffused state; but the artifact has proved useful in several ways, notably in tracing the history of the granulosa derivatives.

Besides the granulosa lutein cells and the blood-vessels, the corpus luteum contains cells of another type, whose origin has been traced by the author to the theca interna of the Graafian follicle. Corpora of the eighth to the tenth day always contain a few distinct clumps of theca interna cells in their original position about the periphery of the corpus luteum and along the vessel-bearing septa which pass radially inward where the follicle-wall was infolded by collapse at ovulation. Many others of the theca cells, however, have wandered among the granulosa lutein cells, from which they can usually be distinguished by smaller size (diameters of 10 to 25 micra), by a more deeply staining cytoplasm, which is often densely packed with minute regular vacuoles, giving a characteristic foamy appearance, and (in osmium preparations) by the presence of plentiful fat globules which vary greatly in number and size (fig. 13, pl. 2, *th. l. c.*). The theca lutein cells often have squared or irregular outlines, fitting into the interstices between the swollen rounded surfaces of the larger granulosa lutein cells (fig. 12, pl. 2, *th. l. c.*).

The tissue is held together by a framework of reticular connective fibrils which, as illustrated in figure 4, plate 1, from a preparation by Bielschowsky's method, form a dense network about all the lutein cells. In view of the apparent

scantiness of fibroblasts of the usual type, the presence of so complete a reticulum gave cause for surprise until it was found (Corner, 1920) that here, as in a number of other organs, the capillary endothelial lining is the source of the reticulum. As the capillary bed is so complex that every cell in the gland is in contact with a blood-vessel, it is not difficult to understand the great density of the reticulum.

RETROGRESSION OF THE CORPUS LUTEUM.

It is a fact of the greatest importance that the changes, just summarized, which lead up to the formation of the corpus luteum, take place with equal completeness, whether or not the sow has been impregnated. It is not possible to distinguish the corpus luteum of pregnancy from that of unfertilized ovulation during the first 2 weeks after discharge of the ova, nor can the observer determine, from the appearance of two specimens of equal age, which of them was destined to further growth in size and a 4 months' span of activity, and which to immediate retrogression.

The time of degeneration of the corpus luteum of unfertilized ovulation can be determined with fair accuracy, for the data, though few, are in complete accord with each other. Seven animals of our series were taken 15 or more days after an observed œstrus but before another ovulation had occurred, and all of these contained retrogressing corpora lutea, while all those killed earlier showed no sign of degeneration; 2 of the 7 had begun a second œstral period and thus gave a further check upon the time relations. It is upon the fourteenth or fifteenth day, then, that a sudden change overtakes the structure which has been so elaborately erected. Within 2 or 3 days the diameter of the corpus has decreased from 8.5 or 10 mm. to 6 mm.; its color has changed from the pink of an active capillary circulation to the whitish tone of scar-tissue, and its texture has much increased in firmness and toughness. Microscopic examination (fig. 6, pl. 1; fig. 14, pl. 2) shows the change to have been so rapid as almost to defy comparison with the previously existent conditions, and exact analysis of what has happened is thus rendered difficult. An example of the suddenness of the break-down is given by one of the animals, killed on the fifteenth day, in which some of the corpora were degenerated while others were intact, but in one corpus there was a patch of advanced degeneration surrounded by unchanged tissue.

The most striking feature of the change is the disappearance of the granulosa lutein cells. In the sows whose ovaries show the least degeneration many of these cells can still be distinguished in various stages, from a fair state of preservation to almost complete degeneration (fig. 14, pl. 2), but in other animals of this period nothing remains but vague vacuolar spaces containing many pycnotic nuclei and nuclear fragments, where but a few days before were closely packed masses of cells, among the most imposing of the animal body. Since these cells in number and bulk formed by far the greater part of the corpus luteum, it is obvious that their breakdown alone will account for the decrease in size and for the relatively greater density of the retrograding corpus. Another result of the degeneration is seen at the periphery of the corpus luteum, where the former sharp demarcation

between lutein tissue and the surrounding capsule of connective tissue is now obscured (fig. 6, pl. 1). In one or two of the animals there are great numbers of polymorphonuclear leucocytes in the tissues and also mononuclear wander-cells of the macrophage type, no doubt to serve the purpose of clearing away cellular débris.

The fate of the blood-capillaries is a matter of special interest in view of what has already been said about the capillary endothelium as the source of reticular connective-tissue fibrils. Deprived of much of their circulatory function by abolition of the granulosa lutein cells, the capillaries for the most part collapse, with nuclear degeneration in some cells, but remain *in situ* with the reticulum. There is at first no increase in the total amount of connective tissue, though the decrease in bulk of the corpus luteum crowds the fibrils into smaller space; but as retrogression proceeds (fig. 5, pl. 1) the reticulum gradually becomes denser and thicker until it gains an appearance like that of collaginous tissue, which is so characteristic of the corpora albicantia (fig. 7, pl. 1). Whether in this change the endothelial cells are gradually replaced by inwandering fibroblasts, or themselves retain the fibroblastic function, can not be said with certitude, but there seems to be no *a priori* reason to doubt the latter possibility, in view of what we know of cellular dedifferentiation in general. It may at least be definitely stated that the fibrous tissue of the retrogressing corpus luteum is laid down *in situ*, for the Bielschowsky preparations make it clear that there is no direct growth of fibers from the surrounding ovarian stroma into the corpus.

It remains to discuss the fate of the cells of the third type, which the writer has described as theca lutein cells. These seem, surprisingly enough, to survive the blow which destroys the granulosa lutein cells; for among the débris and the collapsed capillaries, and also in occasional clumps at the periphery, are found numerous cells which, by reason of their usually angular or elongated shape, foamy cytoplasm, and wealth of osmium-staining fatty material, can hardly be deemed other than the theca lutein cells (figs. 15 and 16, pl. 2, *th. l. c.*). In one or two animals the osmium preparations show numerous globules of blackened fatty substance as large as 30 to 40 micra (represented, of course, in ordinary sections by vacuoles) which seem to lie in some of these same cells, suggesting that they, too, are temporarily affected by the process of degeneration, to the extent of partial fatty degeneration. When after a few days the nuclear fragments and the vacuolar spaces left by the degenerated granulosa derivatives have disappeared (fig. 15, pl. 2), the lipoid-laden cells are more clearly seen, enmeshed in the scar-tissue, where they persist for weeks, acquiring even denser stores of yellow-pigmented fat (fig. 8, pl. 1).

By the time of a new ovulation the corpora lutea have diminished to 6 mm. diameter, at the mid-intercœstral period to 4 mm., and by the second ovulation (*i. e.*, when their own age is about 6 weeks) to 3 or 2 mm. Küpfer (1920) also finds that they can be traced through a second intercœstral period. After this they can still be recognized in sections for at least another cycle of ovulation, but finally become so obscure that they can no longer be certainly distinguished from atretic follicles. It is interesting to note that the two specimens of retrogressing corpora

lutea of pregnancy (known to be of the seventh and tenth days after parturition) which were briefly mentioned in the previous paper (Corner, 1919) are quite similar in microscopic appearance to those of the non-pregnant sow of the same relative period of retrogression; it is very likely, therefore, that there is no difference in the mode of degeneration of the two types of corpora lutea.

What is given above is the first attempt, except for that of Leo Loeb (1911*a*), dealing with the guinea-pig, to describe the histological details of retrogression of the corpus luteum from specimens of known age, and in view of the somewhat unorthodox result one is disinclined to enter upon a discussion of its general bearing, except in a tentative way. It may fairly be said, however, that should the writer's views of the structure of the corpus luteum, especially with regard to the fate of the theca interna, be borne out by other work, they will afford a means of reconciling one of the old disagreements of ovarian histology. It has often been pointed out that in cellular arrangement the theca interna of atretic follicles rather closely resembles the corpus luteum. Both tissues consist of large lipoid-laden cells supported by a reticular framework, with a rich capillary blood-supply. In the later stages of retrogression the two tissues are indeed confusingly alike, and, in the writings of Paladino (1879), Clark (1898), and many others, this fact has been one of the mainstays of the theca-origin theory of the corpus luteum. This idea (in connection with the view now held by nearly all investigators, that the so-called "interstitial cells" found in some mammalian ovaries are derived from the theca interna of atretic follicles) has been the basis of various attempts to correlate these three elements of the ovary, such, for instance, as that of Ancel and Bouin (1909).

Sobotta (1896), however, with his clear-cut demonstration, now generally accepted, that the granulosa layer of the follicle takes a very important part in corpus-luteum formation, could not agree to any statement of resemblance between the two types of follicle-derived tissue, and so expressed himself, when participating in a general discussion of the fate of the corpus luteum at the 1908 meeting of the Anatomische Gesellschaft. According to his description of events, in the corpus luteum the granulosa persists and the theca interna is used up in the production of connective tissue, while in atresia, of course, the granulosa breaks down and the theca proliferates.

Following, on the other hand, the present writer's account of formation of the corpus luteum, and assuming that atresia folliculi, which has not yet been adequately studied in the pig, is the same here as in other species, it will be seen that the two processes differ in their course, but not greatly in their end-stages. The granulosa cells, which degenerate in atresia, degenerate also in retrogression of the corpus luteum, after their temporary metamorphosis into granulosa lutein cells; while the theca interna cells, which by our account do not revert to fibroblasts when they enter the developing corpus luteum, persist alike in the degenerating corpus and in the atretic follicle. In this way the cells of the membrana granulosa, derived presumably from the "germinal epithelium," are associated with the ovum rather than with the soma, and those of each follicle run their course and disappear when their own particular ovum leaves the body, either by degeneration

or by parturition; whereas the theca interna cells, presumably derived from the ovarian stroma, behave as if morphologically part of the somatic ovarian tissues, only temporarily bound up with the fortunes of the ovum. It is to be hoped that this conception may be tested by work on other species.

Text-figure 2 gives, in diagrammatic self-explanatory form, a picture of the ovarian cycle as we have described it in the foregoing pages.

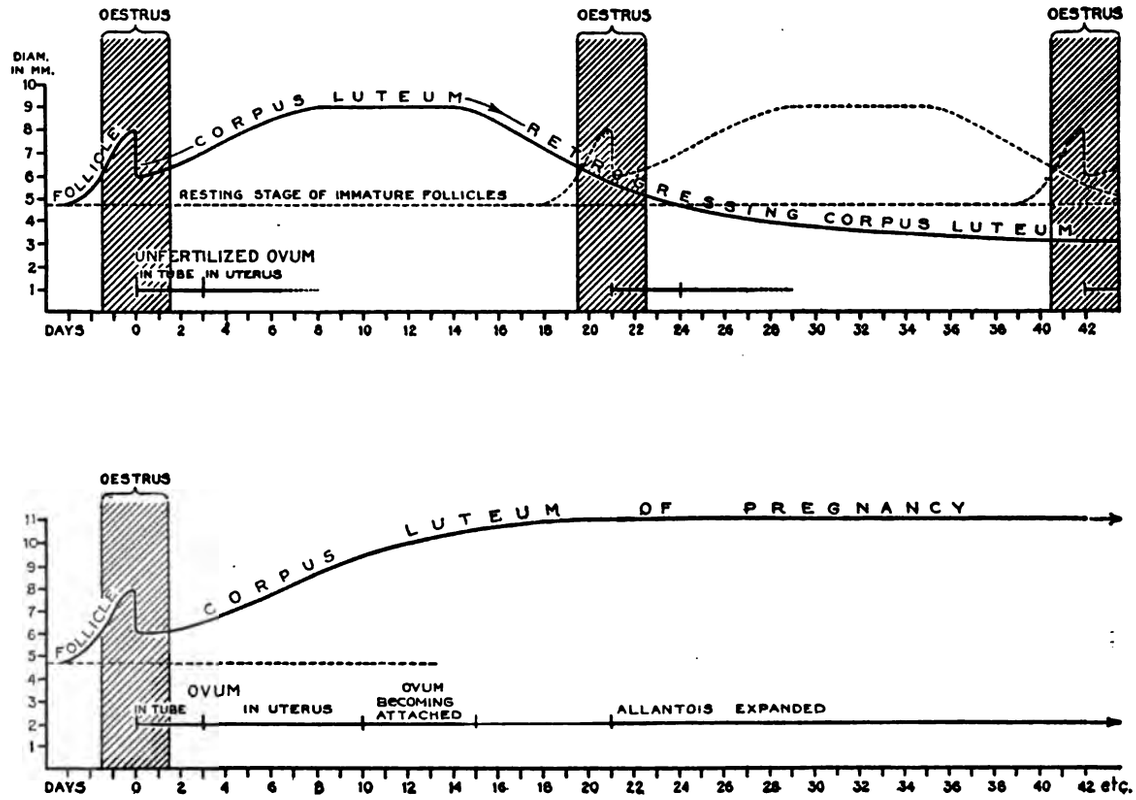


FIG. 2.—Diagram showing in graphic form the relations between œstrus, ovulation, the development of the corpus luteum, and the progress of the ova in the sow.
Above: events of the average cycle of 21 days in the non-pregnant sow.
Below: events of the first weeks of pregnancy.

III. UTERUS.

GENERAL DESCRIPTION OF THE SOW'S UTERUS.

During the period of sexual maturity the sow's uterus undergoes regular alternations of structure, correlated with the ovarian cycle, which are continuously in progress and are varied in their course only by the occurrence of pregnancy. These alternations involve changes in the degree of surface folding of the mucosa, in the morphology and dimensions of the surface epithelium, in the dimensions of the cells of the superficial gland tubules, in the division rate of all the epithelial elements, in the number and kind of wander-cells in the subepithelial portions of the stroma, and in the amount of fluids in the interstices of the stroma. In the following pages these changes will be followed in detail.

The general structure of the cornual portions of the sow's uterus is illustrated in plates 3 and 4. There is a lining epithelium resting upon a stroma of connective tissue, and this in turn is seated directly upon the muscularis, which, as usual in tubular forms of the uterus, is disposed in two layers, the inner circular and the outer longitudinal. Into the stroma pass a large number of glands having openings in the epithelium.

The epithelium is fundamentally of a simple one-layered type, but (as we shall see) the columnar arrangement is greatly modified at one period of the cycle, so that some histologists (for instance Schmaltz, 1911) have even described it as "many-layered." We may suspect that there is a good deal of incompleteness in the descriptions of the uterine epithelia of other mammals, due, as in this case, to lack of an understanding of the cyclic changes.

The glands open into the uterine lumen by means of tubules which run more or less directly, with but little branching and slight tortuosity, until they are well into the stroma, when they begin to branch rather freely and to exhibit marked tortuosity. In this way the mucosa is divided into two fairly well marked layers, the superficial zone containing but few glands and the basal zone densely packed with glands so twisted and involved that in sections they are cut across in every imaginable way. There is a slight difference between the cells lining the glands of the two zones; those of the superficial tubules are higher, measuring usually 15 to 25 micra in height as against 12 to 18 micra in the glands of the basal zone. There is thus a greater proportion of cytoplasm to nucleus in the cells of the superficial tubules, and they look larger and clearer than those of the smaller and deeper-lying glands. Furthermore, the lumina of the superficial tubules are usually about twice as wide as those of the basal glands, as might be expected from the probability that many of the latter are drained by a few of the superficial tubules. There are no permanent shorter glands or crypts, as in the uterus of the dog and other carnivores.

There are no ciliated cells in the surface epithelium of the sow's uterus, but cilia begin to appear immediately within the necks of the glands (fig. 17, pl. 2), and may be found throughout the glands. They are better observed in the larger superficial tubules, not only because they are more easily seen in the wider lumina, but also because they are larger, stouter, and more numerous here. One may

roughly guess that in this region one-fourth of the gland cells are ciliated. In the basal zone the ciliated cells seem fewer and the cilia are slighter and less obvious because packed into a narrow lumen (fig. 18, pl. 2, *a*); but when by chance a dilated gland is found they can be seen to good advantage (fig. 18, pl. 2, *b*). If a small piece of the fresh uterine mucosa is snipped off and flattened under a cover-slip, the cilia may be seen beating within the glands. It was by this method, and in fact with tissue from a sow, that the cilia of the uterine glands were first discovered by Nylander in Leydig's classroom (Leydig, 1852).

There is no evidence to indicate that there is other than a serous secretion from either glands or surface epithelium. There are no goblet cells in the uterine epithelia of the sow, and careful microchemical tests for mucin in smaller masses give negative results. Equally negative were the results of an effort to demonstrate unsaturated neutral fats by means of osmium tetroxide. The statements of Wegelin (1911), that there is a cyclic variation in the amount of glycogen demonstrable in the human uterine mucosa, suggested a similar study of the sow's uterus, especially since we had already observed glycogen in the fetal membranes in early pregnancy; but no glycogen could be demonstrated, either by Best's carmine method or by iodine after alcohol fixation, in any part of the uterine mucosa of the non-pregnant sow at any stage of the cycle.

The stroma of the uterine mucosa is no more nor less than a rather gelatinous or fluid-infiltrated areolar connective-tissue, through which course the glands, blood-vessels, lymph-vessels, and nerves, and which contains in the meshes of its fibers the various cells of areolar tissue, namely, fibroblasts, macrophages ("clasmatocytes"), plasma cells (often very numerous, but varying without reference to the cycle), and the various leucocytes. There is a slight condensation of the stroma just under the surface epithelium, forming a narrow subepithelial connective-tissue zone, of which the most superficial fibroblasts are flattened against the epithelium to form a basement membrane.

The blood-vessels (fig. 19, pl. 3) pass through the muscularis, giving off branches to plexuses in and between the muscular layers, and then form a network of large channels near the base of the stroma. From this network long loops ascend toward the lumen, to end in a delicate capillary plexus in the subepithelial tissue, just below the epithelial cells.

THE UTERUS DURING ŒSTRUS.

(Figure 20, plate 3; 25, 26, plate 4.)

During the days of œstrus the uterine epithelium has a total thickness of 25 to 30 micra. As shown in the figures, it is not obviously columnar, but presents an arrangement which is deceptively suggestive of stratification. The cells are so closely compressed together laterally, and at the same time have attained so low a form, that they are rather irregularly packed and the nuclei thus appear to be arranged in several layers. On careful study many of the cells appear to extend from base to surface of the epithelium, but

Ova maturing in the Graafian follicles, or discharged and in passage through the tubes. Earliest stages of the corpora lutea.

others seem to be impeded by their neighbors from reaching the free surface. The cells are small, since there is a relatively low proportion of cytoplasm to nucleus; so that, taking all these criteria together, a histologist coming upon such a tissue for the first time might perhaps class it among epithelia like those of the urinary bladder rather than among the secretory types of epithelial tissues.

Three details are especially to be noted at this stage: First, mitotic figures are very numerous in the epithelium, in some specimens occurring as often as 1 in every 50 nuclei. The epithelium, therefore, is actively proliferating. Second, a contrary process is also indicated by the presence here and there of phenomena of degeneration; at the base of the epithelium there are points where two or three cells have become vague of outline, with chromatolysis of the nuclei, so that a small vacuole is formed in which lie a few nondescript nuclear fragments or granules (fig. 26, pl. 4, *v. d.*). This degeneration is, as we shall see, merely the latter stage of a phenomenon which sets in slightly earlier than the period of œstrus. The same may be said of the third fact of especial interest, namely, the presence, in large numbers, of neutrophilic polymorphonuclear leucocytes in the subepithelial connective-tissue, and even of a few which are embedded in the epithelium, presumably making their way into the lumen (figs. 25, 26, pl. 4, *p. m.*).

During œstrus the stroma of the uterus, in mature animals, is very edematous, so that the interspersed cells are widely spaced (fig. 20, pl. 3).

There are a few mitoses in the cells of the superficial gland tubules, but none in those of the deep glands. A curious feature is the presence, in some of the gland cells, of highly chromatic extra-nuclear granules, reaching a diameter of 1 to 2 micra. The nuclei of cells possessing these granules are usually of normal appearance; but I have tentatively considered the granulation as a degeneration phenomenon affecting a few cells of the glands. It is seen only during the œstrous period.

TRANSITIONS DURING FIRST WEEK AFTER OVULATION.

(Figure 27, plate 4.)

During the first week after ovulation we see the onset of changes which finally effect a striking alteration in the form of the surface epithelium. The individual cells at first merely grow larger, so that the layer which they form is further piled up to a thickness reaching 35 to 50 micra. The vacuolar spaces indicative of degeneration disappear by the end of œstrus, and by the time the eggs have passed into the uterus there are no more polymorphonuclear neutrophil leucocytes to be seen in the epithelium or subepithelial stroma. Mitotic divisions of the surface epithelium continue to be very numerous until the end of this period, when they cease altogether, not to be seen again until just before the next œstrus. About the time of their cessation there is a further change in the morphology of the surface epithelial cells, which have been growing in height and which at last are somewhat suddenly ranged into a simple, high columnar epithelium, which will be described in the next section.

Ova in transit through the Fallopian tubes; then in the uterus, where they degenerate about the seventh or eighth day. Had they been fertilized the ova would now be passing through the stages of segmentation and blastocyst formation. The corpora lutea are in process of formation.

There is also a wave of mitotic division in the gland cells, which does not begin, however, until the ova are about to pass into the uterus (3 or 4 days after ovulation). In the superficial gland tubules the mitoses cease simultaneously with those of the surface epithelium, about the sixth or seventh day, but in the basal glands a few mitotic divisions may be seen a day or two longer, even after degeneration of the ova and establishment of the high columnar surface epithelium.

Eosinophil polymorphonuclear leucocytes, which are always present in small numbers in the uterine stroma, especially in the more superficial portions, undergo a great increase in number during the first week after ovulation. Unlike the neutrophils of the œstrous stage, the eosinophils do not invade the epithelium, but remain in groups about the vessels and glands of the superficial zone.

There is a marked reduction of the edema of the stroma at the conclusion of œstrus, so that the connective tissue and the structures passing through it are again condensed into somewhat smaller compass.

STAGE OF EIGHT TO TEN DAYS AFTER OVULATION.

(Figures 21, 24, plate 3; 28, plate 4.)

The surface epithelium now presents a striking contrast to its former appearance, for its cellular elements are tall and narrow and ranged in simple columnar form. The relative proportion of cytoplasm to nucleus is greatly increased and the nuclei generally occupy a central position. The cells vary in height from 20 to as much as 45 micra, and are arranged in such a way that the surface formed by their free ends is not smooth but wavy, giving rise to little hillocks, between which are depressions or pseudocrypts (fig. 21, pl. 3). As we shall see, this arrangement aids in giving the mucosa, in the gross specimen, a soft and velvety texture. The whole appearance is now that of an actively secreting epithelium.

The ova have degenerated and disappeared. Had they been fertilized they would now be large blastodermic vesicles, not yet fixed to the uterine mucosa. Corpora lutea fully organized.

At this stage one notes particularly well a detail which has often been commented upon by observers of other species, including the human, and which is seen at all stages of the cycle, namely, the presence between the normal epithelial cells of others which are compressed laterally, have darker-staining cytoplasm, and usually compressed pycnotic nuclei (fig. 28, pl. 4, *i. c.*). They have been called "Stiftchenzellen," "cells with pycnotic nuclei," "intercalar cells," etc., and have been variously interpreted. I can see no reason to doubt that view which holds them to be degenerated cells in the act of extinction. Here and there one also sees small cells of uncertain provenance, with dense, round nuclei, which seem to have intruded themselves into angles at the bases of the tall epithelial cells upon the basement membrane.

At this time the invasion of the more superficial parts of the stroma by eosinophil leucocytes (described in last section) is at its height, as shown in figure 24, plate 3.

The cells of the superficial gland tubules now undergo a slight enlargement. From the seventh to the tenth day of the cycle they measure from 18 to 30 micra, at all other times from 15 to 20 micra. Likewise, the cells of the basal glands gain a little in height, exceeding their usual limit of 10 to 15 micra and gaining a

dimension of 15 to 20 micra. This enlargement of the gland cells is due to an increase in the relative amount of cytoplasm, so that they also gain an appearance as of active serious secretion. It should be mentioned here that at no time are there any marked changes in the morphology of the glands like those described in the human uterus by Hitschmann and Adler (1908) and others.

STAGE OF THE TENTH TO THE FIFTEENTH DAY.

(Figure 22, plate 3; 29, plate 4.)

The stage of high columnar epithelium is succeeded about the tenth day after ovulation by another phase, which is characterized by a further modification of the surface epithelium. This consists of a reduction of the cells to a low columnar form, measuring from 15 to 20 micra, and by the extrusion (from the surface of each cell) of cytoplasmic processes ranging from 3 to 8 micra in height, which will be more readily comprehended by study of the illustrations than from verbal descriptions. In some preparations the appearance is as if the free ends of the cells were frayed and eroded; in others the processes are rounded or pointed, and sometimes they might almost be taken for poorly fixed or agglutinated cilia, except for the absence of the basal granules which mark all true cilia in the uterine glands. (Compare fig. 29, pl. 4, with fig. 17, pl. 2.)

No ova in the uterus; had they been fertilized the embryos would now be gaining attachment to the uterus. Corpora lutea in a fully developed stage until degeneration sets in on the fifteenth day.

We shall have occasion to return, in a later section, to the consideration of this very peculiar change of the epithelium. There appear to be no similar observations on record except those of Hitschmann and Adler (1908), who describe (in the late-interval and premenstrual stage of the human uterus) epithelial cells with frayed-out surfaces, and of Geist (1913), who illustrates, also in the human premenstrual stage, rounded protuberances very much like those of the pig. Geist considers that the protuberances consist of a secreted substance passing out of the cells into the lumen; but as far as our specimens are concerned there seems to be no reason for thinking them other than a modification of the superficial cytoplasm.

Coincident with the lowering of the height of the epithelial cells, there is a disappearance of the complex hillocky arrangement of the surface. The gland epithelium now returns to its usual size and the excess of eosinophil leucocytes disappears from the subepithelial stroma.

STAGE OF THE FIFTEENTH TO THE TWENTIETH DAY.

(Figure 23, plate 3; 30, plate 4.)

During the few days previous to a new ovulation the surface epithelial cells become so low (15 to 20 micra) that in some preparations they are of cuboidal form, hardly higher than their own nuclei. The surface protuberances diminish in height and finally disappear as the cells once more begin to be arranged into a pseudostratified epithelium. This stage marks what might be called the completion of the cycle; in the pig it is of brief duration, but in the sheep, where there is a long anœstrous period, the uterine mucosa apparently remains in such a resting stage during the 10 months or more of interval.

During the latter part of this stage a new group of follicles is prepared for ovulation. The corpora lutea are degenerating.

During the last days before the onset of œstrus, however, a few mitotic figures are found in the surface epithelium and at the same time there are numerous points of vacuolar degeneration of the epithelial cells. The most remarkable event of this stage is the assembling of large numbers of neutrophil leucocytes in the subepithelial stroma. In some of the specimens (fig. 23, pl. 3), the leucocytes are seen swarming out of every arteriole in the superficial stroma and passing toward the epithelium; a little later they are arranged along the bases of the epithelial cells and a few are to be found passing through toward the lumen. An œdematous state of the stroma also sets in toward the end of this period, and thus, by a combination of all these changes, the uterine mucosa is brought up to the œstrous stage, at which we began the description.

To conclude this cursory description of the uterine cycle, it may be added that the histological changes are not without effect on the general appearance and texture of the uterine mucosa. An opened uterus taken during the œstrous period is paler than at other times, with a firmer and at times slightly gelatinous inner surface (due to the œdema); while during the rest of the cycle, and perhaps especially from the ninth to the tenth days, the mucosa is pink or red, soft, and velvety. There is also a periodic change in the external dimension of the uterus, for, by plotting the circumferences (at the mid-points of the cornua) of the uteri of our 22 mature sows of similar age, in the order of their cyclic stages, a significant curve is obtained which is directly correlated with the degree of œdema of the stroma. In other words, the uterus is slightly larger just before and during the period of heat, because the stroma is then thickened by œdema. Caution must be used, however, in the interpretation of measurements and gross appearances as seen in specimens obtained at random at the slaughter-house, for there are numerous possibilities of uncontrollable variation, especially in the amount of bleeding of the carcases and in the ages of the animals. Some are taken very young, even before the first œstrus, and therefore long before the uterus has attained full development. A marked state of œstrous œdema does not seem to occur at the earliest heat periods.

For the same reason I have refrained from dogmatic statements on a point of some interest—namely, whether there is a postœstrous hypertrophy of the glands, such as has been described, for instance, by Keller (1909) in the dog. Study of our 22 animals of known age and comparable size, though suggesting a positive answer, does not suffice to settle this question, since there are wide variations in the number of glands, and one is further confused by the difference in fluid-content of the stroma. It is difficult to compare specimens in one of which the glands are widely spaced and the stroma thick, while in the other the glands are densely packed in a narrow space.

Another uncertain matter concerns the numerical relations of the epithelial cells. There is a period of very active proliferation of both surface and glandular epithelium, but no time of widespread destruction. One is forced to assume for the present that the postœstrous wave of mitosis is compensated for by the sporadic degeneration of epithelial elements, which must be very frequent if the compressed cells (page 138) are, as suggested, in a moribund or degenerated state, and by the loss of cells due to the vacuolar degeneration of the preœstrous and œstrous periods.

THE UTERINE MUCOSA DURING THE EARLIEST WEEKS OF PREGNANCY.

(Figures 31, 32, 33, plate 4.)

It now becomes a matter of great interest to compare the histological state of the uterus during the earliest weeks of pregnancy with the cyclic alterations just described in the non-pregnant animal. The only available information on this point is given in brief form by Assheton (1906) in his description of the ungulate placenta. It has therefore seemed so important to make renewed observations on this subject that a series of early pregnant uteri has been assembled (by the methods outlined above, pages 123-124), including ova undergoing segmentation in the tubes and uterus, morulæ and early blastocysts, and implanting embryos in the shield and earliest somite stages. These have been obtained from the butcher and are therefore without exact data as to age; they have been arranged into a series by comparison of the corpora lutea and the stage of the embryos. The probable ages in days have been estimated from the data of Assheton (1898) on the rate of development of the pig embryo and from our own studies of the development of the corpus luteum (Corner, 1919).

Examination of these very early pregnant uteri gives a result which is as important as it is simple. The same histological changes are found during the first 15 days of pregnancy as during the 15 days following an œstrus without copulation. By the seventh day the œstrous epithelium has passed into the high columnar stage, with hillocky arrangement of the surface; mitoses have ceased in the epithelium but are numerous in the glands; eosiniphil leucocytes are present in large numbers in the subepithelial stroma. Thus by the time the ova have developed into large spherical blastocysts (eighth and ninth days) the uterus can in no wise be distinguished from a non-pregnant uterus 8 to 10 days after ovulation. (Compare fig. 28 with fig. 31, pl. 4.) Two uteri containing wrinkled vesicles of age estimated at 11 days show low columnar epithelium with smooth surface, diminution of the hillocky arrangement of the epithelium, and few eosinophils. By the fourteenth or fifteenth day, when the chorion is in contact with a large part of the mucosa, the low columnar or cuboidal cells are covered with the curious frayed or rounded protuberances which we have seen to be characteristic of the non-pregnant uterus at the same time after ovulation (fig. 32, pl. 4). These changes of course affect the more distant crevices and angles of the mucosa as well as those areas where the chorion is applied to the uterine surface. Where the chorion has been separated from its attachment to the epithelium, as inevitably occurs over large areas during fixation, it may be seen that the trophoblast is pitted and roughened by contact with the irregular surface of the epithelium (fig. 33, pl. 4).

After this stage the identity of the histological processes in pregnant and non-pregnant uteri is at an end. At a time when the non-pregnant uterus is again undergoing precœstrous changes (18 to 20 days) the pregnant uterus presents even lower epithelial cells and greater roughening of the cellular surface, so that Assheton speaks even of a degeneration of the epithelium at this time (fig. 33, pl. 4). The cells do not die off, however, but on the contrary again become of medium or high columnar type, and so persist, as is well known, throughout pregnancy. The stage

of low epithelium with surface protuberances or roughening may quite as plausibly be considered not a degenerative phase, but one of physiological significance, in some way. (See page 143.)

PREVIOUS ACCOUNTS OF THE UTERINE MUCOSA OF THE SOW.

Certain previous contributions to the cyclic anatomy of the pig's uterus deserve consideration at this point. Givkovitch and Ferry (1912) attempt a direct comparison between the changes of the human menstrual cycle and those of the pig's uterus. They describe, without giving the time relations, four stages of the corpus luteum: formation, full development, early and advanced involution; and they correlate with these stages four steps in the condition of the uterine mucosa, which they call prehyperæmic, hyperæmic, posthyperæmic, and interval. Such a division does not disagree with our present description, although we have not seen all the histological changes mentioned by Givkovitch and Ferry. It is to be regretted that their preliminary note has not been followed by a definitive account.

Stegu (1912) interested himself chiefly in the question of distribution of the uterine cilia. He had 60 animals at various stages of the cycle and made studies of the fresh mucosa in all of these with fixed and sectioned preparations of 15 of them. As a result of this work he has given the best account of the external features of œstrus that the present writer has seen; he speaks of ovulation during heat; hints at the œstrous œdema of the uterine mucosa, and mentions the degeneration of certain epithelial cells at this time. Being anxious to contrast œstrous with definitely non-œstrous uteri, he had but one animal killed between the end of œstrus and the tenth day thereafter; in this specimen he observed the hillocky arrangement of the epithelium, with crypt-like depressions intervening, which we have found to be characteristic of this stage.

IV. SUMMARY AND DISCUSSION.

It remains to consider how far our results have justified the hope, with which we began, that the reproductive cycle of the sow might prove illuminating in proportion to its simplicity. We have found, first, that there is a regular periodic chain of events in the ovary; beginning with rupture of the follicle during œstrus, the corpora lutea attain complete organization about the seventh day and hold their full development until the fourteenth or fifteenth day. We have seen that this interval is just long enough to cover the period during which the embryos are becoming attached, should pregnancy result from the ovulation. If no pregnancy occurs, atrophy of the corpora lutea begins about the fifteenth day.

In the uterus we have seen a correlated cyclic alternation. During œstrus there is a characteristic state of the uterine mucosa very similar to that which has been described by Loeb (1914) and by Stockard and Papanicolaou (1917) in the guinea-pig, and very fully and accurately by Long and Evans in their forthcoming monograph on the rat. During the time of formation of the corpora lutea the epithelium, glands, and stroma undergo a series of changes which attain their height about the same time that the corpora become solid. From the eighth to the tenth day the uterine epithelium presents the appearance of active serous secretion, and then its cells lose height, subside to a low columnar or cuboidal form, and become marked by cytoplasmic protrusions or roughenings on their free ends. After the fifteenth day, when the corpora lutea begin retrogression, there is a slow reversion to the œstrous type of structure. A key to the understanding of these changes is found in the fact that an exactly similar histological progression occurs during the first two weeks of pregnancy, no doubt serving as a mechanism to permit attachment of the embryos. Recalling the elementary type of implantation in this species, one is tempted to form the simple, perhaps crude hypothesis that the uterine epithelium by its serous-secretory stage provides for flotation of the delicate embryonic vesicles and thus facilitates their migration and spacing in the relatively extensive uterine cavities (see Corner, 1921); but during the period of attachment the mucosa is, on the other hand, rendered glutinous by the peculiar surface roughening of the epithelial cells, in order to assist in attachment of the chorions.

Whatever the functional value of these histological details, the reader will agree that the processes described in this paper strongly suggest that the underlying fact of the uterine cycle of the sow is an upbuilding of the mucosa, presumably under control of the corpora lutea, for the purpose of successful implantation. Each act of ovulation is thus accompanied and followed by uterine changes, which either go on to placenta formation or (in the absence of embryos) subside once more, as do the corpora lutea, in preparation for a new ovulation.

Such a general conception has long since been suggested by the work of a number of investigators, including that of Hitschmann and Adler (1908) on the human uterus, L. Loeb (1911*b*, 1914) on the guinea-pig, Keller (1909) on the dog, Ancel and Bouin (1910) on the rabbit, and Hill and O'Donoghue (1914) on *Dasyurus viverrinus*. These writers did not see the changes exactly as we have seen them in

the pig, nor do they agree in all particulars among themselves, though most of them describe a wave of cell-division in the epithelium and considerable growth of the glands and stroma in the days following ovulation. The discrepancies of detail are no doubt partly due to the fact that each of the animals used for these studies is a law unto itself, both as to anatomy of the reproductive organs and as to outward expression of the cycle. Preparation of the pig's uterus for the diffuse non-deciduate placentation of this species may well involve changes somewhat different from preparation for the elaborate implantation of the guinea-pig and man. Clearly, much remains to be done before satisfactory generalizations can be established.

Tabular synopsis of the ovarian and uterine cycle.

	Ovary, non-pregnant animal.	Ovary, pregnant animal.	Unfertilized ova.	Fertilized ova.	Non-pregnant uterus.	Pregnant uterus.
Œstrus	Follicles enlarging, then ovulation, then earliest stages of the corpus luteum, (For illustrations, see Corner, 1919.)		Maturing, in follicles then in tubes.	Maturing in follicles then in tubes. Conjugation. Early segmentation.	Œstrous type of epithelium; epithelial vacuolar degeneration (later stage); mitoses; migration of leucocytes; œdema of stroma. (Pl. 3, fig. 20; pl. 4, figs. 25 and 26.)	
Fourth to seventh day.	Developing corpora lutea. (For illustrations see Corner, 1919.)		In uterus; degenerate about 7th to 8th day.	Pass into uterus; segmentation and early blastocyst stages.	Transitions to high columnar epithelium; continued mitosis in epithelium; invasion of stroma by eosinophil leucocytes. (Pl. 4, fig. 27.)	
Eighth to tenth day.	Full development of corpora lutea. (Pl. 1, figs. 3 and 4; pl. 2, figs. 10, 12, and 13.)		.....	Unattached blastocysts	High columnar epithelium giving impression of active serous secretion on surface and in glands; no mitosis in epithelium; numerous eosinophils. (Pl. 3, figs. 21 and 24; pl. 4, figs. 28 and 31.)	
Tenth to fifteenth day.	Full development of corpora utea.		.....	Gaining attachment to uterus	Low columnar epithelium with altered surface of the cells. (Pl. 3, fig. 22; pl. 4, figs. 29 and 32.)	
Fifteenth to twentieth day.	Retrogression of corpora lutea. Enlargement of new follicles. (Pl. 1, figs. 5 and 6; pl. 2, figs. 11, 14, 15 and 16; later stages of retrogression, pl. 1, figs. 7 and 8)	Continued growth and persistence of corpora lutea. (For illustrations see Corner, 1915.)		Implanted; expansion of allantois and vascularisation of membranes.	Very low epithelium, becoming "pseudo-stratified" with renewed degeneration tion, mitosis, and migration of leucocytes. (Pl. 3, fig. 23; pl. 4, fig. 30.)	Columnar epithelium, to which chorion is applied. (Pl. 4, fig. 33.)

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DESCRIPTIONS OF PLATES.

Comparison of the plates will be facilitated by use of the tabular synopsis, page 144.

PLATE 1.

FIG. 3. Section of the corpus luteum about 10 days after ovulation, showing fully developed granulosa lutein cells. Bouin's fluid, Mallory's connective-tissue stain. Photograph. ×210. (Figure 12, plate 2, shows higher magnification of the same preparation.)

FIG. 4. Corpus luteum about 13 days after ovulation, showing reticulum stained by Bielschowsky's silver-impregnation method. Photograph. ×210.

FIG. 5. Corpus luteum (degenerating), about 17 days after ovulation, showing increase in reticulum. Bielschowsky. Photograph. ×210. (Same animal as fig. 15, plate 2.)

FIG. 6. Corpus luteum, degenerating, about 20 days after ovulation. Bouin, iron hematoxylin. Photograph. ×25.
a, blurring of delimitation between lutein tissue and the capsule, as described in text.

FIG. 7. Corpus luteum in well-advanced degeneration, about 4 weeks after ovulation, illustrating complete disappearance of the granulosa lutein cells. Bouin, iron hematoxylin. Drawing. ×80.

FIG. 8. Corpus luteum (degenerate) about 7 weeks after ovulation, showing numerous fat-bearing cells presumably derived from the theca interna. Osmium tetroxide fixation without other stain. Photograph. ×150.

PLATE 2.

FIG. 9. Ovary of a sow in œstrus. Natural size. *m. f.*, mature follicles.

FIG. 10. Ovary of a sow about 8 days after ovulation. Natural size.
c. l., corpora lutea of the latest ovulation (8 days old). (See fig. 3, plate 1, and figs. 12, 13, plate 2.)
r. c. l., retrogressing corpora lutea of the next previous ovulation (8+21 days). *i. f.*, immature follicles.

FIG. 11. Ovary of a sow about 17 days after ovulation. Natural size.
c. l., corpora lutea of the latest ovulation (17 days old), now retrogressing. (See fig. 14.)
f., Graafian follicles enlarging preparatory to the next ovulation, due in about 4 days.

FIG. 12. Corpus luteum about 10 days after ovulation. Bouin, Mallory's connective-tissue stain. ×700.
gr. c. l., granulosa lutein cell.
th. l. c., theca lutein cell.

FIG. 13. Corpus luteum about 10 days after ovulation. Osmium tetroxide fixation without additional staining, to show fat. ×700. *gr. l. c.*, granulosa-lutein cell. *th. l. c.*, theca lutein cell.

FIG. 14. Corpus luteum in early degeneration about 17 days after ovulation, showing collapsed capillaries and degenerating lutein cells. Bouin, Mallory's stain. ×700.
cap., collapsed capillary blood-vessels.

FIG. 15. Corpus luteum in slightly later degeneration about 17 days after ovulation (not the same animal as fig. 14). Most of the cellular débris has disappeared. Bouin, Mallory's stain. ×700.
th. l. c., persistent cell, presumably a theca lutein cell.

FIG. 16. Corpus luteum in early degeneration about 17 days after ovulation (same animal as fig. 14). Osmium tetroxide for fats. ×700.
th. l. c., persistent fat-bearing cells, presumably theca lutein cells.

FIG. 17. Uterus, showing mouth of gland, to demonstrate that cilia are present in the glands but not on the surface epithelium. Bouin, iron hematoxylin. ×330.

FIG. 18. Uterus, showing ciliated epithelium in glands lying at the base of the mucosa, near the muscularis. Bouin, iron hematoxylin. ×330.

PLATE 3.

FIG. 19. Section of uterus showing blood-vessels. Ink injection, carmine stain. Photograph. ×10.

FIG. 20. Uterus during œstrus. Bouin, iron hematoxylin. Photograph. ×10.

FIG. 21. Uterus about 8 days after ovulation. Bouin, iron hematoxylin. Photograph. ×10.

FIG. 22. Uterus about 15 days after ovulation. Bouin, iron hematoxylin. Photograph. ×10.

FIG. 23. Portion of uterine epithelium with subjacent arteriole about 17 days after ovulation, showing migration of polymorphonuclear neutrophil leucocytes from the blood-vessel. Bouin, carmine. Photograph. ×500.
pm., polymorphonuclear leucocytes.

FIG. 24. Portion of uterus about 7 days after ovulation, showing accumulation of eosinophil leucocytes in the stroma. Photograph. ×120.
eos., eosinophil leucocytes.

PLATE 4.

Sections of sow's uterus at various periods of the cycle. Fixed in Bouin's fluid, cut at 5 micra, and stained with iron hematoxylin. Drawn at uniform magnification of 600 diameters.

FIG. 25. Uterus during œstrus.
p. m., polymorphonuclear leucocyte en route through the epithelium.
mit., mitotic figure in dividing cell.

FIG. 26. Uterus during œstrus.
v. d., vacuolar degeneration.
p. m., polymorphonuclear leucocytes at base of epithelium.

FIG. 27. Uterus (non-pregnant) about 6 days after ovulation. *mit.*, mitotic figure in dividing cell.

FIG. 28. Uterus (non-pregnant) about 8 days after ovulation. *p. s.*, pseudocrypt. *i. c.*, intercalar cell.

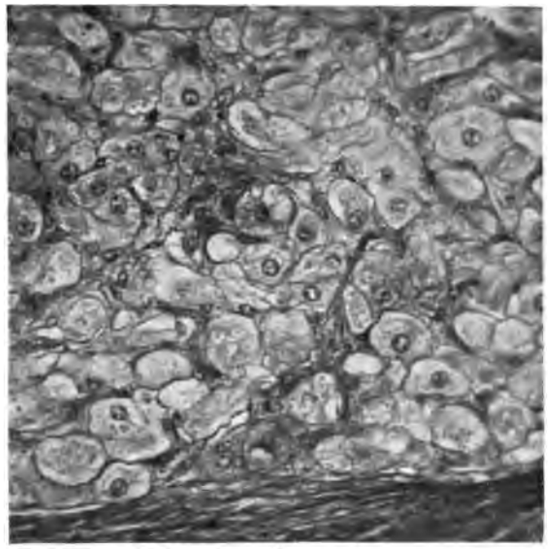
FIG. 29. Uterus (non-pregnant) about 11 days after ovulation.

FIG. 30. Uterus (non-pregnant) about 16 days after ovulation.
mit., mitotic figure in dividing cell. *v. d.*, vacuolar degeneration.

FIG. 31. Uterus (pregnant) about 8 days after ovulation.

FIG. 32. Uterus (pregnant) 14 to 15 days after ovulation.

FIG. 33. Uterus (pregnant) 18 to 20 days after ovulation. *cho.*, chorion of embryo, partly detached.



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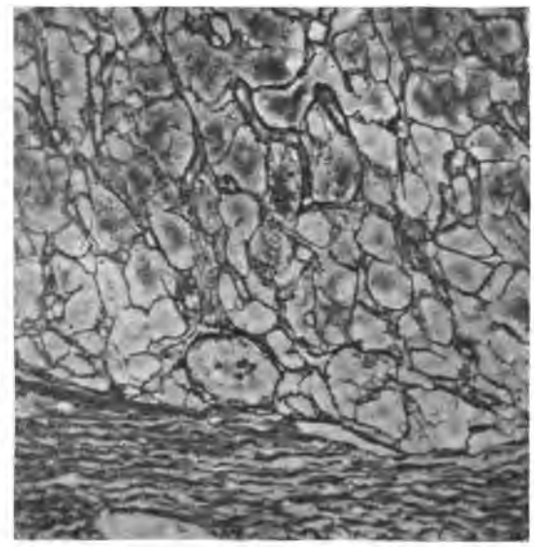

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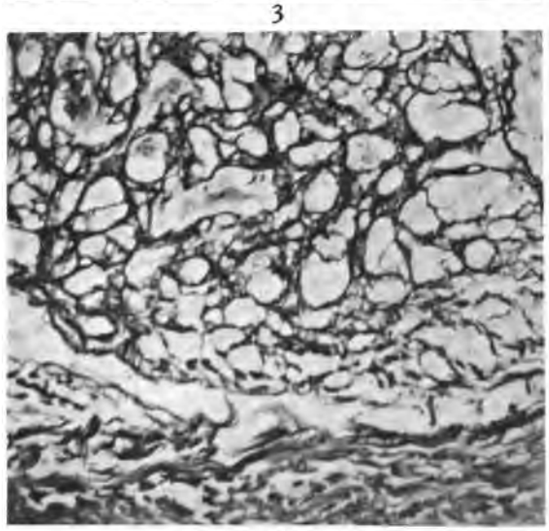

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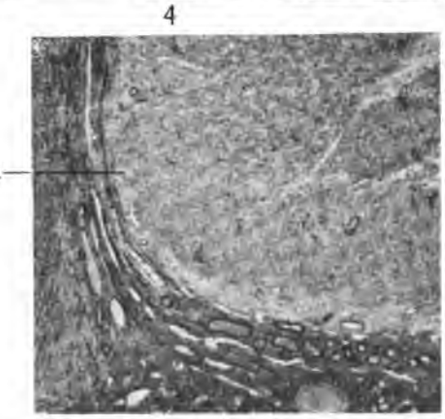

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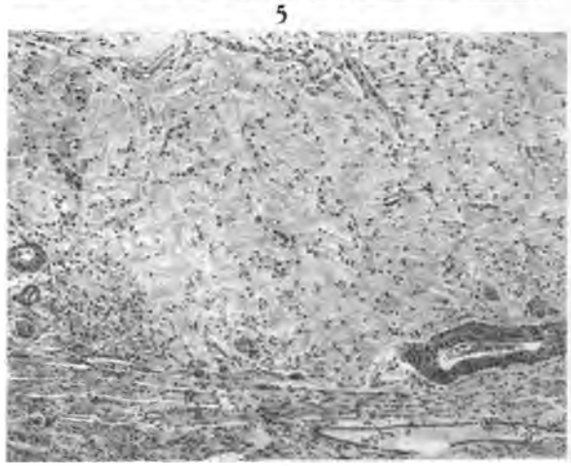

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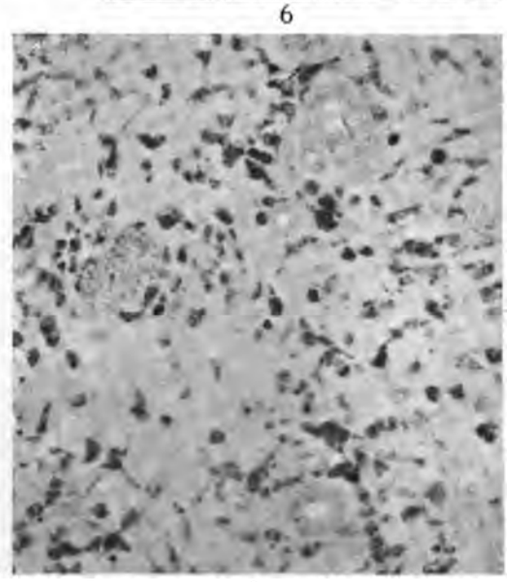

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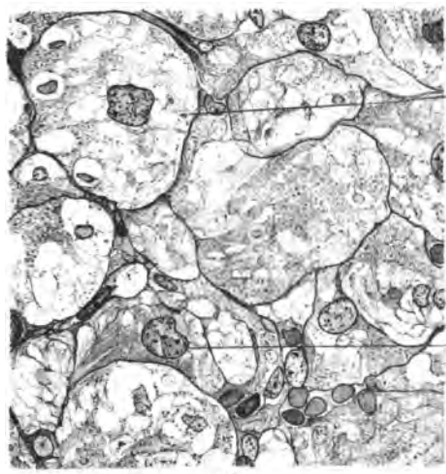

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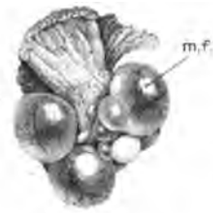

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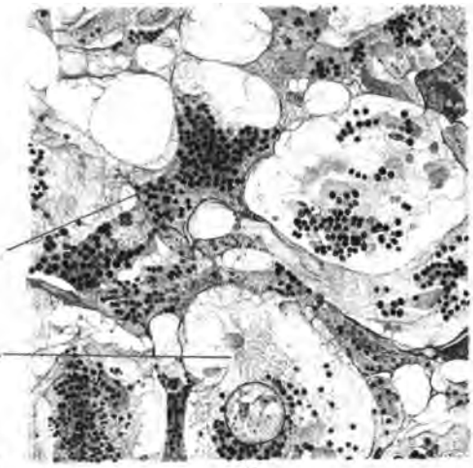

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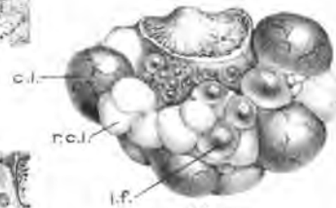

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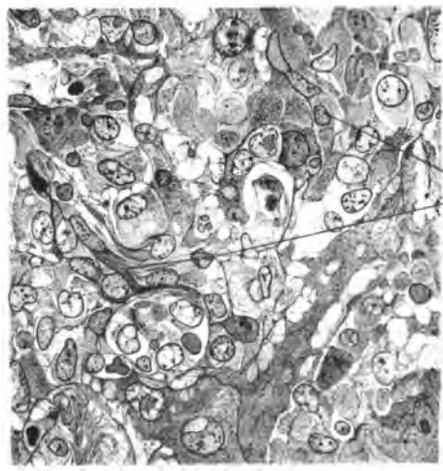

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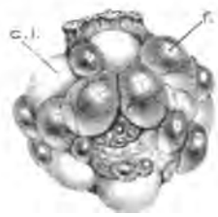

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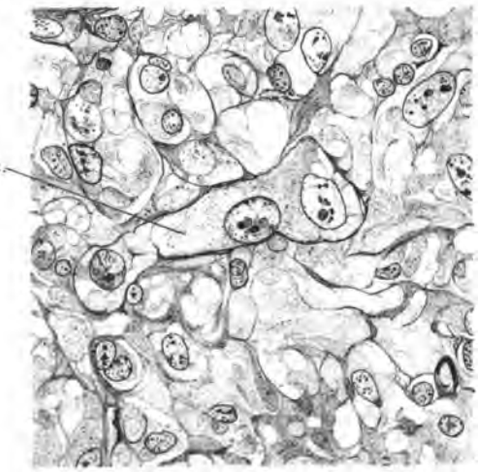

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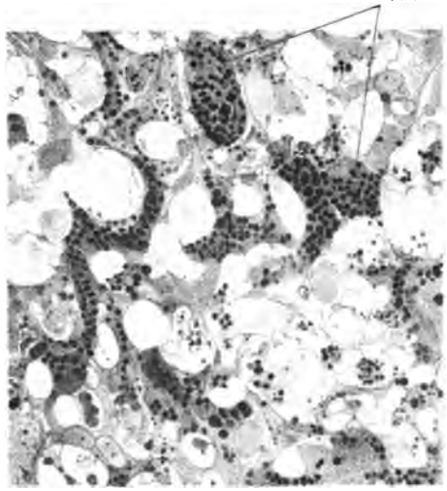

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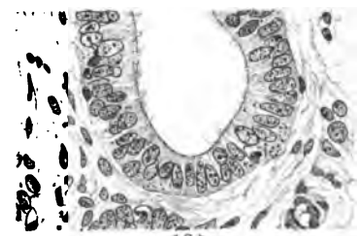

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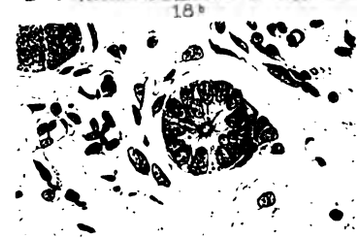

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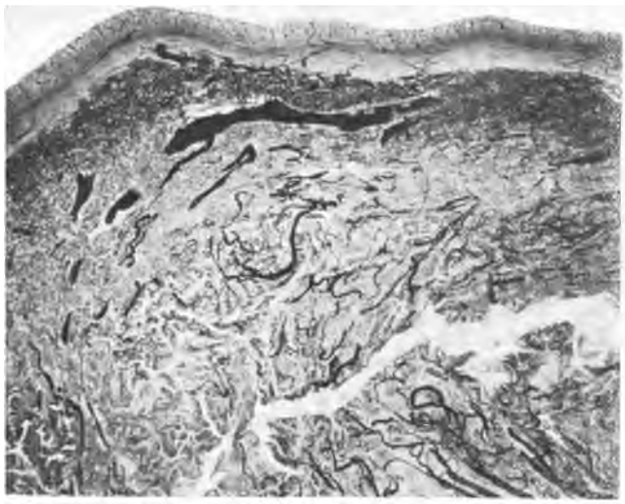

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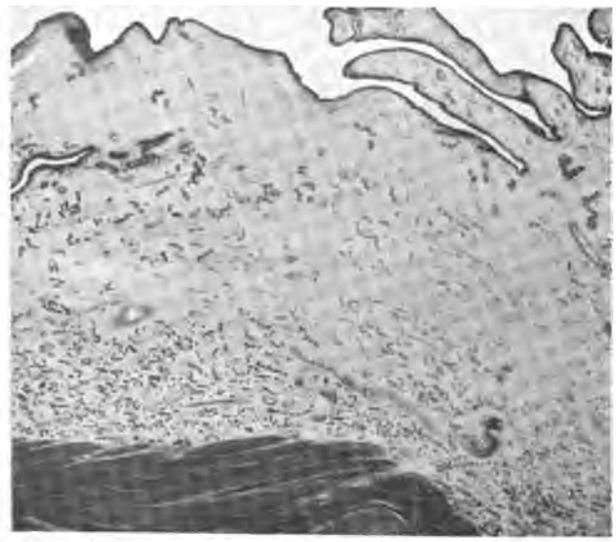

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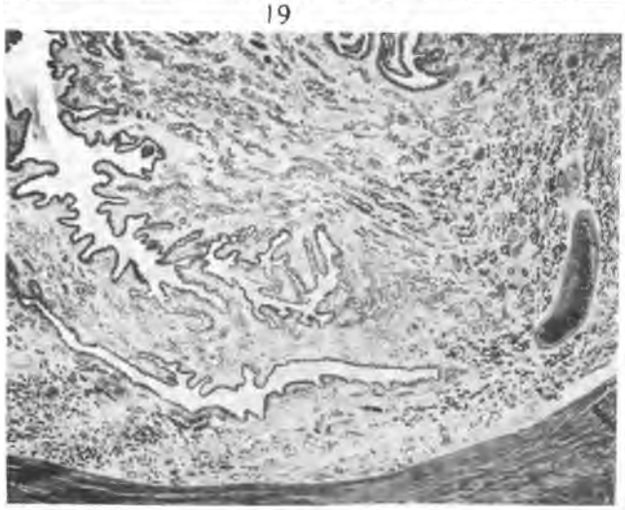

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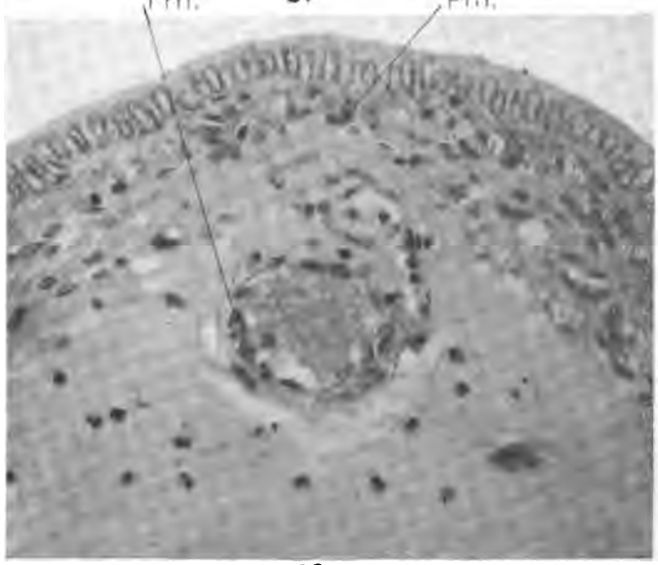

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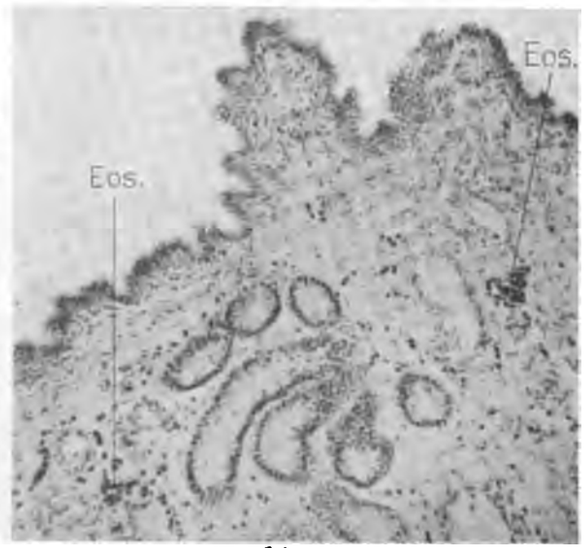

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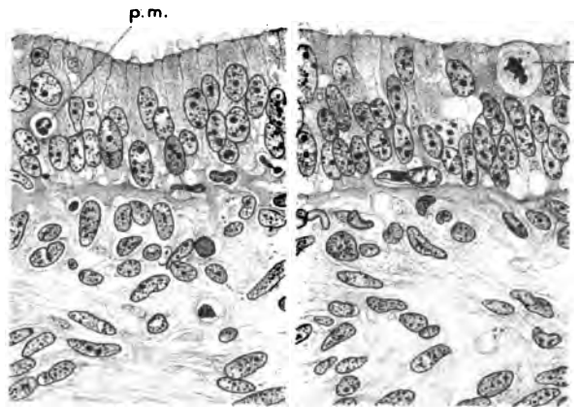

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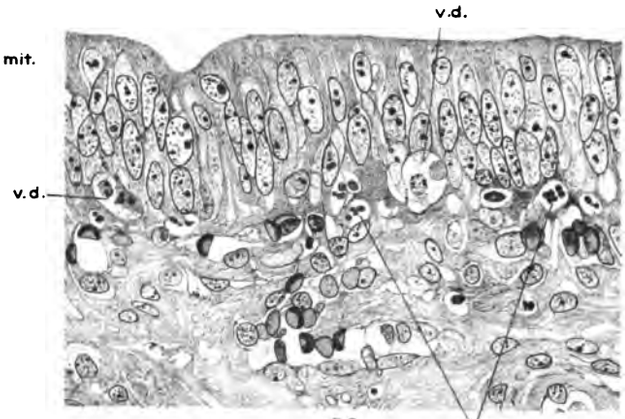

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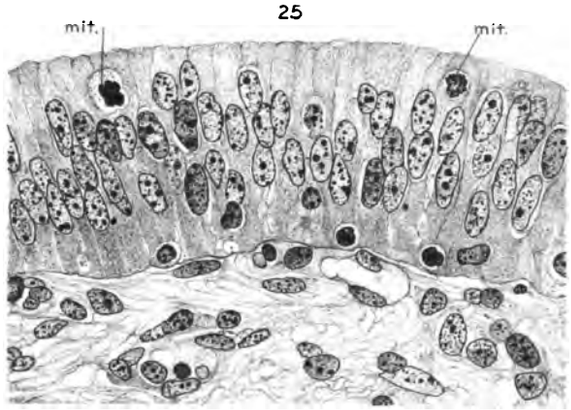

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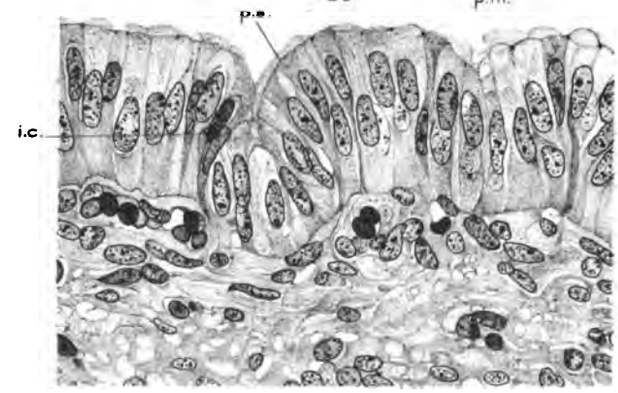

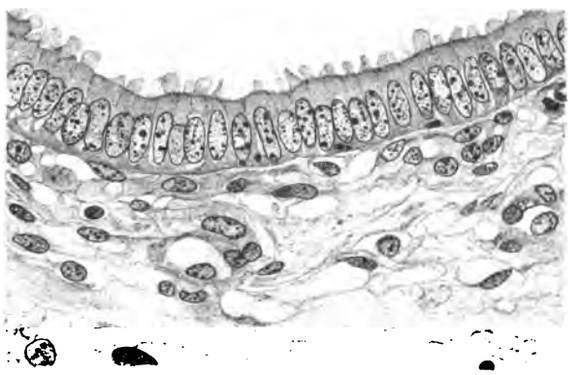

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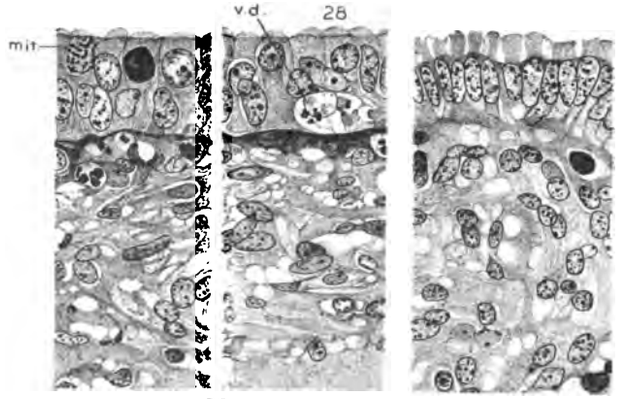

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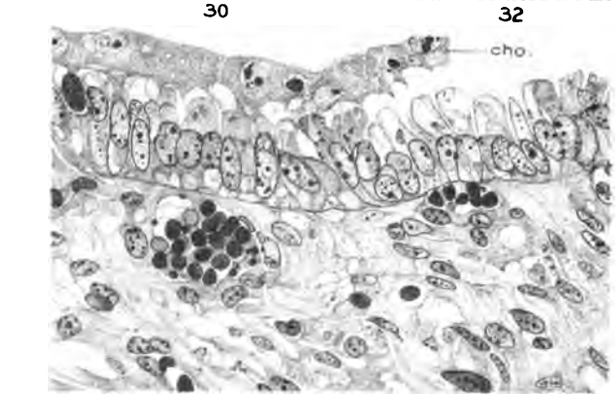

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J. F. Didusch lith. A. Hoen & Co. imp.